INTRODUCTION

Worldwide around 150-200 million people are affected by HCV, involving nearly 3% of the population.\textsuperscript{1-3} According to WHO estimates in 2011, around 3-4 million people are infected by HCV with a mortality of greater than 350,000 every year.\textsuperscript{4} Increased incidence has been seen mostly in Asian countries and Africa.\textsuperscript{1} In Pakistan, 4.8% of the population is affected according to WHO estimates.\textsuperscript{4} According to one large study in 2011,\textsuperscript{5} incidence of anti-HCV positivity was reported to be 0.4-33.7% with predominance of genotype 3 in Pakistan.\textsuperscript{3,5} In 2013, CDC recommended anti-HCV testing as a screening test for population at risk.\textsuperscript{10} Recent detection of DNA/RNA by molecular techniques such as PCR (Polymerase Chain Reaction) allows quantification and analysis of different viral genomes, nucleotide analysis and their resistance to antiviral treatments.\textsuperscript{7} Presence of HCV RNA in the blood indicates ongoing replication of the virus, which is detectable in serum after 1-3 weeks of infection.\textsuperscript{8} WHO recommends nucleic acid testing, which can be done by PCR as the standard procedure of detection of HCV activity. Two types of PCR testing is commonly available, one is a qualitative and the other a quantitative test.

For both the diagnosis and treatment response, HCV is measured by qualitative and quantitative tests.\textsuperscript{6-8} Qualitative test is used for the detection of HCV in the blood stream and also used to assess the virological therapeutic response, assessing active from resolved infections and also for screening the blood of donors. If it is negative at the end of treatment and 6 months later, the probability is that long-term cure has been achieved. The qualitative test uses either PCR or a process of transcription mediated amplification (TMA) method.\textsuperscript{9} In the qualitative test transcription mediated amplification method, RNA is extracted from patient sample and is then analysed by two enzymes which then forms billions of RNA amplicons. These RNA amplicons are detected by hybridization protection assays, which have analytical sensitivity of 5-15 IU/ml for most genotypes.\textsuperscript{6,8} Quantitative analysis is used for accurate treatment planning, for monitoring progression of disease and for assessing antiviral response after treatment.\textsuperscript{5} Real time PCR lower limit of detection is 10-15 IU/ml.\textsuperscript{6} It is essential to achieve (LOD <15 IU/ml) by high sensitive assay HCV RNA for response guided therapy. The sensitivity and specificity of qualitative test is 90-95% and 98%, respectively in comparison to the quantitative test which has a 99% sensitivity and 98-99% specific for HCV.\textsuperscript{8}
Qualitative versus quantitative PCR in establishing response to chronic hepatitis C treatment with sofosbuvir

Qualitative test indicates the presence and absence of viral RNA, and the quantitative test shows the amount of viral RNA present in serum. Both tests are equally specific.

The most crucial step in decision making for treatment is the optimisation of duration of treatment and its early discontinuation.10,11 Qualitative tests are not only reliable and recommended during treatment for response but are also cost-effective, especially in developing countries.

Delay in recognition of infection, high costs of PCR, and high treatment costs have led to poorer detection, especially in developing regions and are factors affecting both economic as well as disease burden on society and are also responsible for prevention and progression of the disease.6

The objective of the study was to determine the cost-effectiveness of qualitative PCR in monitoring RVR in treatment of HCV RNA by sofosbuvir at an area with low socio-economic status.

**METHODOLOGY**

It was a descriptive study, conducted at Memon Medical Institute Hospital, Karachi, Pakistan, from January to September 2016. The study was conducted according to the ethical principles enunciated in the Declaration of Helsinki, after the study protocol was approved by the institutional review board. Informed consent was taken from patients for inclusion in the study. For this study, data of HCV subjects attending outpatients (OPD) was obtained from the computerized hospital management system (HMS). All patients with HCV RNA positive were given treatment with newer agent sofosbuvir. Genotyping was done prior to treatment. The sample size of the study was 104 patients; this group of patients was given sofosbuvir with or without interferon in the outpatient department. The two PCR tests i.e. qualitative and quantitative testing, were compared via Chi-square tests keeping p-value <0.05 as significant. Among these categories, patients are divided as non-cirrhotic i.e. chronic hepatitis C; and cirrhotic, which included compensated chronic liver disease (CLD) and decompensated CLD.

Patients of both genders, above 18 years till 80 years of age having chronic HCV infection and treatment-naïve or treatment relapsers, were included. The exclusion criteria included those who did not give informed consent and pregnant patients. Initially, genotyping and quantitative PCR test was done. During therapy, as per recommendation by American association for the study of liver diseases (AASLD), four times qualitative PCR done for monitoring change; once at the start of treatment, then at 4 weeks that is RVR, then at the end of treatment either 12 or 24 weeks, depending on the type of therapy to see ETR and then 12 weeks after completion of therapy to see SVR. Patients were followed-up till sustained virological response i.e. SVR. Initially, quantitative testing was done and then after completion of therapy, qualitative and also quantitative was done to confirm for eradication.

After the initial PCR showed positive, patients were given either dual or triple therapy, according to genotype. Those patients who were relapsers, non-responders or who had discontinued treatment due to adverse events mainly given triple therapy; while treatment-naïve and cirrhotic patients were given dual therapy. Dual therapy comprises of sofosbuvir 400mg once daily and weight based ribavirin (1000mg daily for <75 kg and 1200mg for >75 kg in two divided doses), and in triple therapy pegylated interferon was added to this regime. Laboratory parameters were followed for treatment related side-effects. The RVR was checked by qualitative PCR and verified by quantitative PCR testings.

The statistical analysis was done through SPSS software 17. The mean and standard deviation was calculated for quantitative variables; and for qualitative variables, frequencies and percentages were recorded. The sensitivity of test was calculated by true positives for HCV PCR by quantitative analysis divided by true positives for HCV PCR by quantitative analysis and false positives. True positives was defined as patients with qualitative PCR not detected, i.e. <15 IU/l and false positive were patients with qualitative PCR not detected, i.e. >15 IU/l. The Chi-square test was applied for comparison of two variables like qualitative and quantitative PCR, keeping p-value <0.05 as significant.

**RESULTS**

In the 106 patients enrolled, the mean age of the patients was 46.40 ±14 years. Overall, 36.8% (n=39) were males and 63.2% (n= 67) were females, with a male to female ratio of 1: 1.7. The age range of the majority of patients between 19-50 years n=62 (58.5%), while remaining were 51-65 years, i.e. 35.8% n=38 and 66-80 years n=6 (5.7%).

There were 86.8% (n=92) patients who were treatment-naïve, 12.2% (n=13) treatment-experienced relapsers. Nearly 94.3% (n=100) belong to genotype 3 while 3.8% (n=4) genotype 1, 0.9% (n=1) genotype 2, and genotype 4 0.9% (n=1).

The patients enrolled included HCV RNA positive patients treated with sofosbuvir for a period of 4 weeks. At the end of 4 weeks, HCV RNA qualitative test was repeated to see the response of treatment, i.e. RVR.

There were n=92 treatment-naïve, and n=13 treatment-experienced relapsers patients. All the patients after treatment with sofosbuvir i.e. n=105 (99.05%) responded to sofosbuvir with successful achievement of RVR after 4 weeks, except one patient who did not achieve RVR, i.e. n=1 (0.9%), and its treatment was then stopped.
The qualitative testing was compared by Chi-square with quantitative assays simultaneously to check sensitivity of qualitative testing which showed 99.05% results with achievement of viral load of <15 IU/ml. The sensitivity of qualitative test was 100%; while the true positive patients were 105, and no false negative results was reported. The p-value after comparison of both qualitative and quantitative was <0.001.

**DISCUSSION**

Sofosbuvir, a newer antiviral drug has altered the situation with regard to HCV cures by pegylated/standard interferon and ribavirin which previously achieved in some 23%-90% individuals. Nowadays, not only sofosbuvir but also other newer oral antiviral agents are associated with complete cure rates of around 99%-100% with or without interferon. The ELECTRON trial has shown 100% SVR with dual interferon-free regime containing sofosbuvir and ribavirin in genotypes 2 and 3 Variations in responses differ slightly among different genotypes and degree of cirrhosis. This study also noted the RVR of 99.05% with sofosbuvir in all genotypes with majority involving genotype 3, except in one case in which RVR was not achieved.

Virological tools are, therefore, required to see treatment response. The standard method of checking virological response is by the quantitative (real time) assays with minimum detection limit of 10-15 IU/ml. According to current recommendations, presence of HCV RNA is usually sought after four weeks of treatment to see RVR, which is also a strong predictor of SVR. SVR shows an eradication of the virus and it is believed to prevent an adverse histological response. During treatment, strict compliance to therapy is important for initial rapid response. In this study, patients were followed for compliance to treatment; and qualitative testing was done simultaneously with quantitative analysis for each patient. The results of both assays were compared showing statistically significant value (p-value <0.001).

From this outcome, it is therefore concluded that in the case of patients treated with sofosbuvir, which has higher rates of success, qualitative analysis can be done in patients for checking RVR.

In this study, all patients in whom RVR was achieved by both methods for detection of HCV PCR, i.e. qualitative method as well as quantitative method, revealed similar results with all the cases showing no HCV RNA and values of <15 IU/ml by quantitative analysis. Different studies have compared different methods of qualitative analysis, e.g. between TMA and COBAS but not much difference between two groups was found. Dalgard et al. found achievement of RVR in 74.8% by COBAS, and 63% by TMA in patients given treatment with pegylated interferon. Qualitative PCR is the most sensitive, cost-effective tool; and negative results are also associated with most probable long-term cure. Although due to contamination through aliquoting or any procedure, false positive test can be reported. However, consistently negative/not detected results indicate complete clearance of this virus and recovery from infections.

**CONCLUSION**

Qualitative analysis is a cost-effective and sensitive method in patients treated with sofosbuvir for confirming RVR, especially in developing countries where low income and absence of third party payers is a big socio-economic burden for the community.

**REFERENCES**


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