ORIGINAL ARTICLE

Outcomes of Hospital Based Therapy to Treat Hepatitis C patients: Study from Local Population of Punjab-Pakistan

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ABSTRACT

Aim: To analyze the outcomes of different interferon therapies used in the Jinnah hospital to treat hepatitis C patients.

Study type: It is a cross-sectional study which was carried out in the Hepatitis Clinic of Jinnah Hospital Lahore from September 2011- June 2012.

Methodology: 301 patients were taken to see the effects of different Hepatitis C therapies used in the Jinnah Hospital Lahore. After getting consent and demographic information of all the patients CBC, LFTs and anti-HCV by ELISA were tested. Quantitative HCV PCR and genotyping were done on the serum of patients. The data was analyzed by using SPSS version 16.

Results: Out of 301 HCV patients, 137(46%) were males and 164(54%) were females. Patients of 19-73 years of age were under study. Five groups of therapies were studied. Although effects of interferon therapies were insignificant but silymarin and polyherbal (Clavazine) had remarkable effects in normalizing ALT levels and gradual decrease in viral load. The herbal antiviral agents may reduce the therapeutic effects of ribazole when used in combination.

Conclusion: Interferon therapies have insignificant effects on viral load. It happens usually due to lowest (price) tender items, short expiry and inadequate storage conditions during transportation which result in loss of their efficacy.

Keywords: Interferon, HCV, PCR, Genotyping

INTRODUCTION

Hepatitis C is the inflammation of liver caused by mainly hepatitis C virus (HCV) resulting in hepatic cell damage[1]. It has been estimated that 200 million HCV infected individuals are in the world. The prevalence of HCV may be different in different regions and various groups of the same community. Approximately, 17 million individuals have been infected with HCV in Pakistan[2]. 32% medical ICU admissions were because of chronic liver disease (CLD) and out of these 69% cases were because of hepatitis C and all the deaths in medical ICU were because of its complications, particularly including encephalopathy and hepatocellular carcinoma (HCC)[3].

Currently, interferon alpha (IFN-α) along with ribavirin is being recommended by clinicians for HCV patients in Pakistan[2]. Interferon of 85 different companies is available in Pakistan but 50% population lives below poverty line and cannot afford to purchase the expensive medicines[4]. About twenty thousand patients got free of cost interferon therapy from "Prime Minister Program for the prevention and Control of Hepatitis" during 2006 -2008. This number is very minute as a large no of patients are still waiting for treatment. [5] The other main problem is that interferon alpha has many side effects thus, about 2-10% of patients have to discontinue their therapy[2].

The current treatment (IFN-α and ribavirin) is neither economical nor fully effective in all patients, the need of the hour is to find out new therapeutic strategies and targets for local genotypes of HCV[6]. Silymarin (SL), a standard combination of flavonolignans mainly (including silybin A and B and isosylibin A and B) extracted from seeds of *Silybum marianum* has been widely used to treat acute and chronic viral hepatits and cirrhosis by improving hepatic functions and enhancing activity of cell membrane because of its excellent hepato-protective effects[7]. Clavazin is a polyherbal medicine and a new therapeutic anti-viral agent used for the treatment of HCV patients, as it has anti-oxidative capacity thus prevents liver fibrosis[8].

The aim and objective of the present study was to assess the outcomes of hospital based therapy used to treat hepatitis C patients.

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MATERIALS AND METHODS

In this cross sectional study, patients were from both the gender males and females, from different age groups usually (19-73 years) and mostly belonged to Lahore and some belonged to surrounding districts. At the time of sampling, a demographic data was collected with complete history of the patients (with possible sources of infection) according to questionnaire.

Materials: The equipments such as Real time PCR system (Rotorgene Model 3000 Australia), ELISA strip reader (Model: SC5123, Germany), vortex mixture (Model: VM 1210, Labnet, USA), micro centrifuge (Model: E 1624, Typ Werk Nr, CE Tokyo, Japan), adjustable micropipettes (Eppendorf Germany) and -20°C freezer, spectrophotometer (Microlab 200 Germany) and other accessories like filter barrier tips (ART, Germany), 2.0 ml screw capped tubes (ART, Germany) and 0.2 ml and 0.1 ml reaction tubes (manufacture Corbett Research Australia) were used. The chemicals including Absolute Ethanol (Merck Germany), Isopropanol (Merck Germany), Formalin and Potassium per magnate (Merck Germany) and RNA mini extraction and amplification kit (Qiagen Germany) were used.

Contents of Extraction and amplification kits: Lyses buffer (560ul / sample), 3.1.2-Carrier RNA, 3.1.3-Internal control, 3.1.4-Absolute Ethanol, 3.1.5-AW1 Buffer (Artus Washing buffer 1) 500ul/sample**,** AW2 Buffer (Artus Washing buffer 2) 500ul/sample, AVE (Artus Elution buffer) HCV Super mix, Mg Sol RT, HCV Standard 1 ($5X10^3$ IU/µl), HCV Standard 2, ($5X10^2$ IU/µl), HCV Standard 3, ($5 X 10^1$ IU/µl), HCV Standard 4, ($5X10^0$ IU/µl) and molecular grade water. All the reagents of the kit were stored at -20°C. Repeated thawing and freezing (>3x) was avoided because they might reduce the sensitivity of the assay.

Plasma Separation: 4cc blood was in collected in EDTA tube. Centrifuge tube at 3000 RPM for 10 minutes to separate plasma and transfer it into two eppendorf cups (one for Real time PCR reaction and second one for Liver Function Tests).

Liver Function Tests: SGPT (ALT) and SGOT (AST) levels were estimated by using spectrophotometer[9]

Total Bilirubin: Bilirubin level was also estimated using jendrassik-grof method photometrically[10]

ELISA method:

Hepatitis C positive samples were confirmed by third-generation commercially available enzyme-linked Immunosorbent assay (ELISA) using Advanced ELISA kits lot no: 2014110602.

PCR (Polymerase chain reaction):

For the confirmation of HCV PCR was done. First of all RNA was isolated according to the instruction of Qiagen (available on their website Qiagen.com) and Qiagen mini Extraction kit was used. After extraction of DNA the amplification of extracted DNA was done. The quantitation was found out using the following formula. The following formula was applied to convert the values determined using the standard curve into IU/ml of sample material:

Result (IU/ml) = {Result (IU/µl) x Elution Volume (µl)} / Sample Volume (ml)

Genotyping: Genotyping was done on Cytoflour, third wave technology USA. The third wave invader assay use cleavase enzymes to recognize and cleave specific structures formed by the addition of two oligonucleotides (an Invader Oligo and a Primary Probe) to a nucleic acid.[11]

Groups of patients with different anti-viral therapies: Five groups receiving different anti-viral therapies were made. First group received one Roferon (Alpha 2a) 3MIU injection thrice a week. In second group, ribazole capsule (400mg) was added (one capsule twice a day). The combination of interferon (thrice a week), ribazole (one capsule twice a day) and silymarin (one encapsulated tablet twice a day) was given to the third group. Only silymarin (one encapsulated tablet twice a day) was taken by the forth group. Clavazin (Polyherbal containing silymarin 200mg, picrorhizin 50mg, glycyrrhizin 150 mg and inulin 75mg) tablet was given twice a day to the fifth group.

Group	Group of therapies	n
1	Interferon	22
2	Interferon and ribazole	188
3	Interferon, ribazole and silymarin	63
4	Silymarin	8
5	Clavazin	20

Patients with discontinuous therapy: 53 patients had to discontinue their therapy because their Hb% became very low after starting interferon injections. Many patients also got more severe side effects like vomiting, diarrhea, severe weakness, and fever. 31 patients were taking interferon only and 22 patients were taking interferon and ribavirin combination therapy.

Statistical analysis: All the data was expressed as mean±standard deviation. The statistical analysis was performed by one way ANOVA using SPSS version 16. A *p* value less than 0.05 was taken as statistically significant.

RESULTS

Hepatitis C is a worldwide health problem and it is the main cause of chronic hepatic disease. This study was done to investigate outcomes of different anti-

viral therapies used in government hospital to treat Hepatitis C patients.1000 patients were screened but out of them total 300 cases were selected for follow up for 06 months. The data was collected from patients referred to Hepatitis Clinic and Molecular Biology department of the Jinnah Hospital during September 2011- June 2012. Patients from both the sexes with different age groups mostly belonged to Lahore and some of its surrounding districts. All the Hepatitis C patients were categorized under following groups:

Table 1: Analysis of variance for the different antiviral therapies used to treat HCV positive patients

Group	Group of therapies
1	Interferon
2	Interferon and ribazole
3	Interferon, ribazole and silymarin
4	Silymarin
5	Clavazin

SOV	Sum of squares	Df	Mean square	F	Sig
Between group	2.501	4	6.254E12	1.633	0.166
Within group	1.130	295	3.829E12		
Total	1.155	299			

Table 2: Descriptive statistics for the different antiviral therapies used to treat HCV positive patients

Group	Group of therapies	Mean±SD
1	Interferon	3.84±1.63
2	Interferon and ribazole	5.18±1.71
3	Interferon, ribazole & silymarin	8.5± 2.31
4	Silymarin	1.02±1.73
5	Clavazin	1.51±3.16

Table 1 described the analysis of variance. It can be seen that there were insignificant effects of different treatment options on viral load as *p* value was >0.05. Although, effects of different therapies were insignificant but silymarin and Clavazin showed marked decrease in viral load as compared to interferon, interferon combined with ribazole and interferon, ribazole and silymarin in combination.

The above table 2 silymarin had maximum effect in decreasing the viral load with the least mean value that was1.02. Clavazin demonstrated gradual decrease in viral load with mean 1.51. Other groups receiving interferon only, interferon combined with ribazole showed mild effect on viral load with means 3.84 and 5.18 respectively. The combination of interferon, ribazole and silymarin demonstrated highest viral load with a mean value of 8.5 which might be due to decreased efficacy of ribazole by silymarin.

Table 3: Analysis of Variance for Liver function Tests (Total bilirubin, ALT, AST) to see the effect of different antiviral therapies on liver

SOV	Sum of squares	Df	Mean square	F	Sig
T Bili					
Between group	.050	4	0.012	2.852	0.024
Within group	1.287	295	0.004		
Total	1.336	299			
ALT					
Between group	1038.918	4	259.729	7.645	0.000
Within group	10022.749	295	33.975		
Total	11061.667	299			
AST					
Between group	2811.824	4	702.956	13.694	0.000
Within group	15143.763	295	51.335		
Total	17955.587	299			

It can be seen from the above table 4.2 (a) that there were significant effects of HCV therapies on liver function tests (total bilirubin, ALT and AST) as the P value was <0.05.

Table 4: Descriptive statistics for Total Bilirubin levels to see the effect of different antiviral therapies on Liver:

Group	Group of therapies	Mean±SD
1	Interferon	0.52±0.06
2	Interferon and ribazole	0.53±0.063
3	Interferon, ribazole & silymarin	0.51±0.071
4	Silymarin	0.58±0.10
5	Clavazin	0.51±0.05

Table 5: Descriptive statistics for ALT levels to see the effect of different antiviral therapies on Liver:

Group	Group of therapies	Mean±SD
1	Interferon	32.0±7.4
2	Interferon and ribazole	33.5±8.2
3	Interferon, ribazole & silymarin	27.0±3.6
4	Silymarin	29.0±7.5
5	Clavazin	25.4±3.4

Table 6: Descriptive statistics for AST levels to see the effect of different antiviral therapies on Liver

Group	Group of therapies	Mean±SD
1	Interferon	28.09± 6.4
2	Interferon and ribazole	27.4± 6.5
3	Interferon, ribazole & silymarin	24.0±3.5
4	Silymarin	24.2± 6.2
5	Clavazin	22.1± 3.1

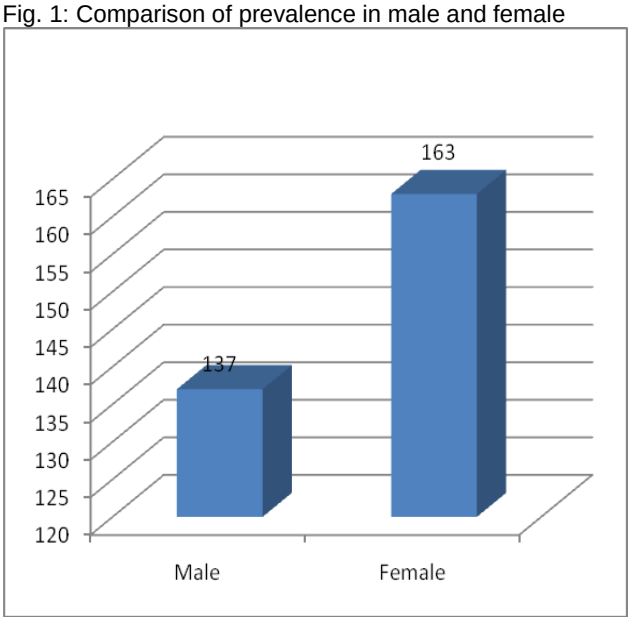
In the above table 4 Clavazin described the remarkable decrease in total bilirubin level with the mean value 0.51mg/dl. Interferon, ribazole and silymarin in combination also showed gradual effect on total bilirubin level with mean value 0.51 mg/dl. The other groups of therapy like interferon, interferon plus ribazole and silymarin alone had high mean value which was 0.52 mg/dl, 0.53 mg/dl and 0.58 mg/dl respectively.

From the above table 5 Clavazin had a remarkable effect on normalizing ALT level with the mean value 22.1U/L. Interferon, ribazole and silymarin, and only silymarin also showed gradual effect on decrease in ALT levels with mean values 24.0 U/L and 24.2 U/L respectively. Interferon and interferon plus ribazole had no remarkable effect on ALT levels and had mean values 28.09 U/L and 27.4 U/L respectively.

In the above table 6 the lowest mean value (25.4 U/L) was shown by Clavazin therapy which had a remarkable effect on normalizing AST level. The second lowest mean showed by interferon, ribazole and silymarin with the mean value 27.0U/L. Interferon, interferon plus ribazole and only silymarin had mild effect on normalizing AST levels with the mean values 32.0U/L, 33.5U/L and 29.0U/L respectively.

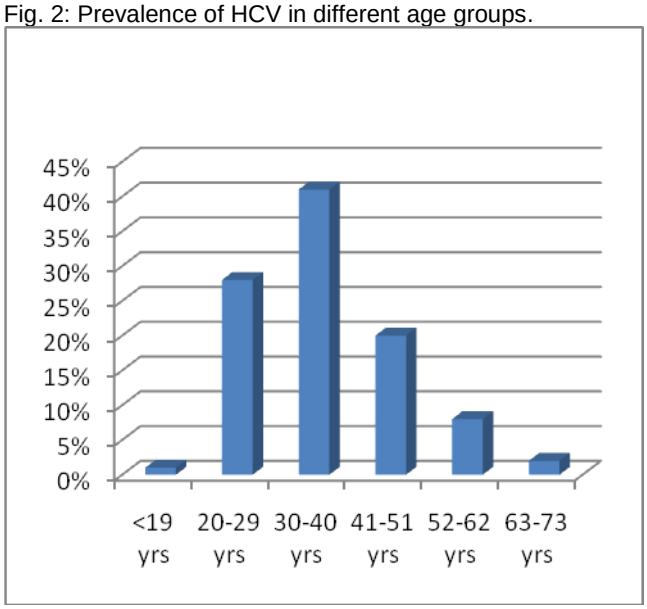
Comparison of HCV in both genders of hepatitis C patients: 137 male and 163 female hepatitis C patients were included in this study. HCV prevalence in male and female liver patients was 46% and 54% respectively.

Fig. 1: Comparison of prevalence in male and female



Prevalence of HCV in different age groups: Patients of all age groups were included in this study and for statistical analysis these were divided into six groups. Group 1 included patients of <19 years age, group 2 included 19-29 year age, group 3 included 30-40 year age, group 4 included 41-51 year age, group 5 included 52-62 year age while group 6 included 63-73 year age. Out of total 300 HCV affected persons of various age groups, the most affected age group was 30-40 years (40.9%) and the second most affected age group was 19-29 years (28.6%).

Fig. 2: Prevalence of HCV in different age groups.



DISCUSSION

The present study showed that all the patients have high ALAT (Alanine aminotransferase) and ASAT (Aspartate aminotransferase) levels which may be due to abnormality in liver function during HCV infection causing damage to hepatocytes. ALT was observed higher in some HCV negative cases because of our hot climate and heavy diet conditions. The other causes of their elevation include fatty liver, viral hepatitis, medication induced hepatitis, autoimmune hepatitis and alcoholic liver disease. ALT was normal in some HCV patients as infection may remain asymptomatic in most of the cases. Different combinations of therapy were used but there was mild or no response on viral load and mild effect on liver function test by interferon combination therapies unless any hepato-protective agent was used. It has recently been reported that addition of herbal medication to ribazole may reduce its therapeutic efficacy; similar findings were observed in the present study with relatively higher viral load in the patients receiving interferon, ribazole and silymarin in combination[12]. The other reasons of

ineffectiveness include purchasing lowest tender items having short expiry, inaccurate storage temperature and unavailability of proper storage conditions during transport.

It has been revealed that in HCV patients receiving silymarin and Clavazin ALT levels come to normal most frequently, similar findings were reported by Jacobs *et al*[13]. The potential mechanisms of action of silymarin include anti-oxidative properties, inhibition of lipid peroxidation, NF-κB activation, mitochondrial injury, P450 activity and inflammation, enhancement of RNA, DNA, and protein synthesis, regulation of cell permeability and immunomodulation[14]. The antioxidant properties of silymarin influence both free radical mediated cytotoxicity and lipid peroxidation in *in vitro* and *in vivo* models of liver disease thus, it has anti-fibrotic effects[15].

The effects of silymarin on hepatic fibrosis have been studied in a gerbil model of iron overload. Animals were given intramuscular iron dextran and either placebo or silybin. There were no differences in hepatic iron accumulation, however silybin treated animals had less hepatic fibrosis as shown by collagen staining. Hepatocytes also had reduced staining for hemeoxygenase suggested reduced oxidative insult[16]. It is obvious that silymarin and polyherbal (Clavazin) which are renowned hepatoprotective agents have fruitful effect in decreasing HCV viral load and also in normalizing liver function tests. A similar type of study was done in which combinations of antioxidants and antiviral agents were used to treat chronic hepatitis C patients and observed that the combination of antiviral and anti oxidant agents could increase general response rate of chronic HCV patients[17].

CONCLUSION

The main purpose of the study was to give a message to common man to get rid of this infection by improving health habits and use such kind of therapy which can be harmless and effective for mankind. The combination of ribazole with herbal medicines reduces its therapeutic efficacy and it also has various side effects. Thus, the clinicians should advise hepato-protective agents like Clavazin and silymarin to treat hepatitis C patients as they have no side effects. There should be use of HCV therapies which have long shelf life and long expiry date. As persons of 20-45 years are at great risk of getting HCV infection so they should be careful about their health and monthly screening for HCV.

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