Sustained Hepatitis C Virus Clearance was Achieved by Honey Based Conservative Management in 35% of Chronic Hepatitis C Virus Patients: A Prospective Cohort Study

MAGD A. KOTB, M.D. and AHMED K. ABDALLA, M.D.

The Department of Pediatrics, Faculty of Medicine, Cairo University

Abstract

Background: Hepatitis RNA viruses include A, B, C and D. They are typically self-limiting by conservative measures, except hepatitis C virus (HCV) that is self-limiting in only 2-5%. HCV causes chronic infection affecting 170 million people worldwide, its prevalence peak in Egypt is 12.1%. About 5%-7% of patients may ultimately die of consequences of HCV infection, e.g. cirrhosis, and hepatocellular carcinoma.

Objective: To assess effectiveness of conservative management in chronic HCV patients.

Methods: In a prospective cohort trial 82 patients with chronic HCV (49 children and 33 adults) were advised honey-based healthy diet, regular exercise and abstinence of unindicated medications. None received concomitant antiviral or interferon therapy. HCV infection diagnosis relies on qualitative HCV RNA diagnosis by nested RT-PCR. Work was conducted in Hepatology Clinic, New Children Hospital, Cairo University, National Egyptian Center for Clinical and Environmental Toxicologic Research, Cairo University & National Cancer Institute, Cairo University during 2008 – 2011.

Results: At enrollment mean age ± standard deviation (±SD) of children was 9.08 years ± 3.5 years (range=3.17-16.75 years, median=9.83 years) and 43 year ± 11.25 years (range=18.5-61 years, median=46 years) for adults. Conservative management was effective in sustaining HCV clearance in 29 (35%). None suffered progression of liver condition. Breakthrough viremia was observed in 8 (9.8%). For those with final lack of virus clearance, HCV load dropped significantly (p=0.007). Sustained HCV clearance correlated positively with younger age (p=0.024), female sex (p=0.037), height attainment in children who were shorter for age at initiation of trial (p=0.002), and negatively with history of anesthesia (p=0.004), previous operative intervention (p=0.000), and did not correlate with initial HCV load (p=0.468). Predictors of response to conservative management await exploration. The HCV sustained clearance was irrespective of initial HCV load.

Conclusion: Conservative management achieved 35% cure of HCV in our studied cohort. It is safe and cost effective. Conservative management in HCV, warrants its trial as a first line of management in chronic HCV patients.

Key Words: Hepatitis C virus – Nutrition – Honey – Drug induced liver injury – Exercise – HCV.

Introduction

HEPATITIS C virus (HCV) infection affects 170 million people worldwide, with a prevalence peak of 12.1% in Egypt. About 5%-7% of patients may ultimately die of consequences of HCV infection, from chronic liver disease, cirrhosis, and hepatocellular carcinoma [1,2].

All hepatidis resulting from hepatotropic (i.e. A, E, D viruses) and non-hepatotropic RNA hepatitis viruses are self-limiting except HCV [3]. Moreover, HCV is not exclusively responsible for liver damage in the HCV infected liver, the synergism of drug induced liver injury and HCV, aggravates and perpetuates HCV, fibrosis and hepatocellular carcinoma [4]. Aflatoxins and alcohol ingestion are well-documented examples of drug induced-HCV synergism [5]. This drug-induced damage is not through increased replication of HCV, but rather due to induction of hepatic fibrosis and liver damage associated with oxidative stress [6,7]. Contribution of drug induced liver damage in HCV might be undermined, especially that purchased self-medications practice is quite common in many countries, e.g. in Jordon, Chili and Egypt it is estimated to be 42%, 75% and 81% respectively [8-10].

Interferon is the mainstay of therapy as monotherapy, as interferon plus ribavirin and as interferon plus ribavirin plus protease inhibitors, with eradication rates of 19-39%, 21-56% and 79% respectively.

Correspondence to: Dr. Magd A. Kotb, The Department of Pediatrics, Faculty of Medicine, Cairo University
It is to be noted that not all subjects suffering from HCV infection receive interferon therapy [11].

Conservative management comprises healthy diet and abstinence from un-indicated medications. Natural honey is part of a healthy diet, as it exhibits antioxidant activity; influences drug detoxification and clearance through an effect on cytochrome P450, namely; CYP3A4 activity but may not affect the activity of CYP2D6 or CYP2C19, protects against chromosomal breakage and helps regeneration [12-14]. In animals it appears that increased CYP3A4 xenobiotic activity requires regular ingestion of honey for several days or more, and that occasional ingestion is unlikely to significantly affect drug plasma concentrations [15].

The present work studied effect of conservative management on spontaneous HCV clearance.

Subjects and Methods

Subjects with HCV were recruited according to guidelines of Declaration of Helsinki and procedures involving human subjects/patients were approved by IRB of National Cancer Institute, Cairo University and by Pediatric Department Committee for Post-Graduate Studies and Research, Faculty of Medicine, Cairo University, Egypt. The study was done during 2008-2011.

Subjects were followed-up at Hepatology Clinics of New Children Hospital, Cairo University Hospital, at National Cancer Institute, Cairo University, and at National Egyptian Center for Clinical and Environmental Toxicologic Research, Cairo University, Egypt.

Inclusion criteria:
- HCV uncomplicated by liver cirrhosis.
- Consent to trial.
- Not receiving standard pegylated interferon/ribavirin.

Exclusion criteria:
- Liver cirrhosis.
- Liver cell failure.
- Hepatocellular carcinoma.

Hepatitis C virus infection diagnosis:
Qualitative HCV RNA diagnosis by nested RT-PCR. The primers from 5'-noncoding region were used for cDNA synthesis and PCR amplification. The primers were as follows: Outside sense primer 5'-GGGGCGACACTCCACCATA-3', outside antisense primer 5'-CCTATCAGGCAGTACCACA-AGG-3', inside sense primer 5'-ACACTCCACCATAGATCACTCCC-3', inside antisense primer 5'CACCACACAC-TACTCG-3'. HCV RNA was extracted using Qiagen RNA extraction kit and copied into cDNA by reverse transcription (RT). PCR productions were visualized on agarose gels stained with fluorescent dye ethidium bromide following electrophoresis [16]. Qualitative HCV RNA assessment was performed using Real time PCR.

HCV typing was not performed, as type 4 is almost always detected in Egyptian patients [17].

No liver biopsy was performed at initial or final visit.

Patients were followed-up for at least thirteen months or a minimum of 6 months after initial negative HCV PCR. HCV PCR was repeated at end of second week, end of second month, then after 6 months and at end of thirteenth month to assess sustained viral clearance, and final outcome.

Response to management was defined according to Ghany et al., [18].

Abstinence of medications, herbs, home remedies and all possible toxins:
All patients were instructed to abstain completely from non indicated medicines and self-purchased medications, exposure to pesticides, fast foods, preserved foods, processed cheese, food additives, foods known to contain aflatoxins (as potatoes, and peanuts), herbs, vitamin preparations containing fat soluble vitamins in excess of recommended daily allowance and hydrogenated oils. Patients who were on the immune-modulatory bile acid ursodeoxycholic acid were advised to gradually withdraw it over 3 weeks prior to enrollment in trial.

Diet:
Recruited subjects were oriented and instructed to adhere to a balanced diet that supplies recommended daily allowance of calories, proteins, carbohydrates and fats guided by age, height, weight and associated medical conditions. Patients were advised to include water-soluble vitamins (B1, B2, B6, folic acid) in recommended daily dose, olive oil, and water within their breakfast. Preparations containing nicotinamide in excess of recommended
daily allowance, or B12 in the form of cyanocobalamine was discouraged.

Patients were advised to adhere to a minimum of 90gm of honey daily. There was no recommendation of a specific type.

**Exercise:**

Regular exercise was encouraged on daily basis. Patients were advised to walk a minimum of 20 minutes daily.

There was no control group receiving standard pegylated interferon, and/or ribavirin.

**Statistical analysis:**

All statistical analyses in this study were conducted using the Statistical Package for Social Sciences version 10 (SPSS, Chicago, IL, USA). Simple frequency, cross-tabulation, descriptive analysis, tests of significance (t-test for parametric data, and X² tests for the study was conducted on 100 HCV patients who accepted to participate, of which 18 patients lost follow-up. To study effect of conservative management on HCV, the patients were involved in a quasi-experimental approach (pre-post administration assessment of the program using the self controlled approach). The minimal required sample size for the study was calculated to be 56 patients based on a required achievement of an acceptable Type I error (alpha error) =0·05 and acceptable Type II error (beta error) =0.2. The expected outcome effect was 30% improvement upon implementation of the program versus a 0% expected improvement without the program.

### Results

Among the enrolled 82 with chronic HCV 49 (59.8%) were children, 33 (40.2%) were adults, 25 (51%) of children were females compared to 11 (33.3%) among adults. At enrollment mean age±standard deviation (±SD) of children was 9.08 years±3.5 years (range=3.17-16.75 years, median=9.83 years) and 43 year±11.25 years (range=18.5-61 years, median=46 years) for adults.

All were diagnosed as chronic HCV patients for more than 1 year prior to enrollment, except one, but exact duration of HCV infection could not be assessed.

Associated risk factors and medical conditions of enrolled subjects are shown in (Table 1).

<table>
<thead>
<tr>
<th>Table (1): Associated risk factors and medical conditions in recruited subjects with HCV.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Risk of exposure to</td>
</tr>
<tr>
<td>Previous blood transfusion</td>
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<tr>
<td>Repeated dialysis</td>
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<tr>
<td>Material used by other HCV infected persons</td>
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<tr>
<td>Operative intervention</td>
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<tr>
<td>Associated Medical Conditions</td>
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<tr>
<td>End stage renal disease</td>
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<tr>
<td>Thalassemia major</td>
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<tr>
<td>Bronchial asthma</td>
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<tr>
<td>G6PD deficiency</td>
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<tr>
<td>Chronic lead poisoning</td>
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<tr>
<td>Fallot tetralogy</td>
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<tr>
<td>Insulin dependent diabetes</td>
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<tr>
<td>Systemic hypertension</td>
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<tr>
<td>Epilepsy</td>
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<td>Obesiy</td>
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<tr>
<td>Hypothyroidism</td>
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<tr>
<td>Malignancy</td>
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<tr>
<td>History of malignancy</td>
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<tr>
<td>Active malignancy</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Juvenile myeloid leukemia</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
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<tr>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>Wilms tumor</td>
</tr>
<tr>
<td>Renal cell sarcoma</td>
</tr>
<tr>
<td>Testicular tumor</td>
</tr>
<tr>
<td>Papillary carcinoma of thyroid</td>
</tr>
<tr>
<td>Brain stem glioma</td>
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<tr>
<td>Neuroblastoma</td>
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</tbody>
</table>

G6PD = Glucose 6 phosphate dehydrogenase deficiency.

At time of enrollment, height of 20 (40.8%) children was below 5th percentile for age, 19 (38.7%) were between 5th and 25th, 2 (4%) were between 25th and 50th, and 8 (16.3%) had normal height for age.

All enrolled subjects had past history of self-medication, namely milk thistle, herbs, pain-killers, antipyretics and local remedies for various durations. History of off-label use of ursodeoxycholic acid was in 19 (23%). Only 49 (59.6%) reported previous intake of indicated medications; chemotherapy in 34 (41.5%) according to protocol therapy of respective malignancy, interferon in 8 (9.7%), and anesthesia in 28 (34%).

Enrolled subjects had a mean initial HCV load of 2,443,865 copies/ml (range=610-10-5x10⁶).

The cohort was followed-up for 17.7±5 months (range=11-35 months). 35 (42.7%) had an initial HCV clearance (initial negative HCV PCR) within a mean duration of 6.7±7.8 months, of enrollment in study (range 2 weeks-28 months). Only 29
(35.4%) achieved sustained HCV clearance (final negative HCV PCR by 13 months or 6 months after initial HCV clearance) (Table 3). One child had a relapse of HCV infection after sustained HCV clearance for 24 months.

Breakthrough HCV viremia (positive HCV PCR in between initial viral clearance and sustained viral clearance) in 8 subjects (6 children and 2 adults) interrupted course of the 29 subjects with sustained HCV clearance. Two children with acute lymphoblastic leukemia suffered malignancy relapse warranting chemotherapy initiation. None reported worsening of general condition. All reported improved agility, better school and work attendance and performance. Forty-seven (95.5%) of children attained between 50th and 95th percentile height for age. Three children entered puberty. A 16 years old male with end stage renal disease on regular dialysis and 2 children with thalassemia did not achieve age appropriate height and 2 did not attain puberty.

Duration to initial viral response among children was significantly longer compared to adults (mean=8.9 months compared to 2.4 in adults, \(p=0.002\)). The 12 children with malignant disease (active or history), had achieved initial HCV clearance within a mean of 11.59 months, compared to 4.5 months in the 13 children with no malignant disease (\(p=0.044\)).

Among the 8 patients with history of previous failure to respond to interferon intake, 2 achieved sustained HCV clearance by conservative management.

Liver transaminases dropped significantly at 11-13 months follow-up visit, but not alkaline phosphatase (Table 2). For those with final lack of HCV clearance, HCV load dropped from initial mean of 3,169,905 copies/ml to 138,582 copies/ml (\(p=0.007\)). Thirty-seven patients of the 47 who did not clear their HCV had a mean drop of 72% of their initial HCV load. Ten subjects had a final mean 224,541 (copies) HCV load, compared to their initial HCV load of 174,856 (\(p=0.509\)). There was no statistical difference between initial HCV load of subjects with sustained HCV clearance, and those who failed to clear their HCV ( \(p=0.468\)).

Initial viral response correlated positively with female gender (\(p=0.036\), follow-up duration (\(p=0.006\)), shorter initial height in children prior to enrollment (0.008), and negatively with history of anesthesia intake (\(p=0.019\)), history of surviving previous malignancy (\(p=0.041\), and previous operative intervention (\(p=0.001\)). Initial HCV clearance did not correlate with initial HCV load (\(p=0.446\)), or age at enrollment (\(p=0.057\)).

Sustained HCV clearance correlated positively with younger age (\(p=0.024\), female gender (0.037), height attainment and catch-up of the children shorter for age at initiation of trial (0.002), and negatively with history of anesthesia (\(p=0.004\), previous operative intervention (\(p=0.000\), and did not correlate with HCV load (\(p=0.468\)).

Failure to clear HCV correlated positive with older age (\(p=0.017\), attaining appropriate height for age prior to enrollment (\(p=0.001\), anesthesia intake (\(p=0.002\), repeated hospital admission (\(p=0.005\), and previous operative intervention (\(p=0.000\), and inversely with duration of follow-up (\(p=0.024\), but not to initial HCV load (\(p=0.0468\)).

Regression analysis defined previous anesthesia as a predictor of lack of viral clearance ( \(p=0.004\)).

In a Logistic Regression Model (95% confidence interval) adulthood (\(p=0.04\), and male gender (\(p=0.035\) predicted lack of HCV clearance, but not HCV load (\(p=0.431\), or associated (active or history of surviving) malignancy (\(p=0.10\)).

Table (2): Initial and final hepatic transaminases, and alkaline phosphatase measured in folds of upper limit of normal.

<table>
<thead>
<tr>
<th></th>
<th>At initial Visit</th>
<th>At initial Visit</th>
<th>(p)</th>
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<tbody>
<tr>
<td>Alanine transaminase</td>
<td>3.35</td>
<td>1.53</td>
<td>0.000</td>
</tr>
<tr>
<td>Aspartate transaminase</td>
<td>2.26</td>
<td>1.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1.2</td>
<td>1.2</td>
<td>0.856</td>
</tr>
</tbody>
</table>

Table (3): Hepatitis C virus clearance in response to exercise, diet and abstinence from medications among 82 enrolled subjects.

<table>
<thead>
<tr>
<th></th>
<th>Children (n=49)</th>
<th>Adults (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of 49 patients</td>
<td>Number of 33 patients</td>
<td></td>
</tr>
<tr>
<td>Initial viral clearance (35 subjects) (42.7%):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>Males</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Females</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Sustained viral clearance (29 subjects) (35.4%):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Males</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Females</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>
Initial HCV clearance | Sustained HCV clearance
--- | ---
45 | 29
35 | 18
47 | 10

Number of enrolled Subjects

Subjects with cleared HCV | Subjects with uncleared HCV
--- | ---
87 (46.5%) | 100 (54.5%)
35 Achieved initial hepatitis C viral clearance | 47 Did not clear Hepatitis C Virus
29 Achieved Sustained Hepatitis C virus Clearance | 6 Did not Achieve Sustained Hepatitis C virus Clearance
37 Achieved 25-99% drop in HCV load | 10 Suffered increase in HCV load
187 were invited to share in trial till September 2009
87 (46.5%) Did not share
100 (54.5%) Shared in trial
18 Dropped out

Fig. (1): Outcome of 82 enrolled subjects with HCV on strict abstinence from drugs and adherence to balanced diet and regular exercise.

Discussion

Conservative management is effective in clearing HCV in only a third of enrolled subjects with chronic HCV:

This original trial provided evidence that 35% of HCV patients have a spontaneous sustained viral response, without side effects. It provides evidence that duration to initial HCV clearance is variable from 2 weeks up to 28 months. It is interesting that long durations are recognized to improve response pegylated interferon plus ribavirin therapy sustained viral response, regardless of genotype [19]. The 35% SVR achieved in our trial is superior to 27% achieved HCV SVR by peginterferon plus ribavirin in genotype 4, which is the genotype almost always encountered in Egyptian population [17] and the 23.9% Hansenula-derived PEGylated (polyethylene glycol) interferon (IFN)-alpha-2a plus ribavirin in children studied in 3 centers in Egypt [18].

Drug induced liver injury is a back stage player in HCV perpetuation:

The study draws attention to HCV-anesthesia synergism as a predictor of HCV chronicity. It is important to stress that HCV infection is a strictly human disease [21], and that HCV infected subjects are at increased risk of exposure to medications, and the long course of disease invites the cultural intake of home remedies, and off-label intake of medications that allege “support” of the liver, which might be hepatotoxic [17,22,23]. It has to be remembered that more than 1000 heterogeneous chemicals, drugs, herbs, foods and remedies, are associated with dose related and idiosyncratic hepatotoxicity [24-26]. The self-medications accessed and purchased practices are undermined sources of exposure to medications in different communities [8-10]. The mechanism of drug induced liver injury in paving way for HCV invasion of liver, or in simple hindering of HCV clearance, or in perpetuation of HCV remains to be studied, and is not within scope of this study.

Predictors of response to conservative management:

Male gender, older age, and history of exposure to anesthesia correlated with lack of HCV clearance, while female gender and younger age correlated with HCV clearance. More insight is needed to fine tune diet planning of HCV infected patients, to characterize predictors of response, to define predictors of synergism of HCV-drug induced liver injury, and to define a management logarithm of HCV patients that addresses clear indications for conservative and for interferon therapy. The HCV clearance was not predicted by HCV load. Specific patterns of time to virologic response were not noted in this trial, probably due to small sample size. Yet viral phase kinetics [27] in response to conservative management might be responsible for the lack of consistent correlation of HCV load to rate of clearance of HCV. Viral kinetics might also be related to resolution of liver injury induced by previous drugs. This might explain why duration
to initial longer viral response among children was significantly longer compared to adults, and the 12 children with malignant disease (active or history), had achieved initial HCV clearance within 11.59 months, compared to 4.5 months in the 13 children with no malignant disease (p=0.044).

Conclusion:
Liver damage due to off-label medications in HCV is preventable, and strict measures should be firmly instituted against self-medications and off-label use of medications in hepatitis patients. Conservative management was effective, safe and cost-effective in clearing HCV in third of patients with chronic HCV. More insight is needed to fine tune diet planning of HCV infected patients and to characterize predictors of response. We encourage conservative management of HCV and foresee that it would downsize the HCV burden in society, in term of cost-effectiveness, hepatic complications of HCV and other complications related to medications used in HCV.

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