Hepatitis C virus is a global infection with an estimated 170 million people affected [1]. It appears that there are endemic strains which have persisted in specific locations for many centuries. These can be readily identified by viral genotype. There have been iatrogenic outbreaks leading to massive spread of specific subtypes in countries such as Egypt (genotype 4A) [2,3]. HCV heterogeneity is huge based on outbreaks leading to massive spread of specific subtypes in countries such as Egypt (genotype 4A) [2,3]. HCV heterogeneity is huge based on infecting individual's body and its very long co-evolutionary history with man. Clinically genotype information on the virus is of major importance in defining response to conventional as well as newer therapies [4].

HVC infection is a major health problem in Egypt [5,6]. The current standard of care for hepatitis C infection is peginterferon/ribavirin, it gives higher sustained response rates in genotype 2/3 infected individuals [7]. But in Egypt the infection is due to genotype 4A for which is less affected by this type of therapy, beside its high cost and numerous serious side effects [8]. This obliged us to search for a safe effective therapy which is less costly as HVC infection is more common in patients with low socioeconomic status. In addition, they are immunocompromised by parasitical infections such as Schistosomiasis and pollution of food by aflatoxins and toxic carcinogenic insecticides.

Since 1983, the blue green algae _Aphanizomenon flos-aquae_ (AFA) has been harvested from Upper Klamath Lake (UKL) in Southern Oregon and marketed as a dietary supplement. It is consumed for its putative beneficial effects including detoxification, increasing energy, elevating mood and loss of weight [9]. Several compounds were extracted from blue green algae including a protein called Cyanovirin-N (CV-N) which appears to irreversibly inactivate diverse strains of HIV virus [10]. CV-N has antiviral activity against HCV as it inhibits HCV entry into host cells at low nanomolar concentrations [11]. Also, blue green algae compounds selectively inhibit the penetrations of enveloped viruses (Herpes simplex, human cytomegalovirus, measles virus, mumps virus, influenza virus and HIV virus) into host cells thereby preventing replications [12-14]. Also, consumption of AFA has rapid effect on the circulation of immune cells in humans [15]. The WHO approved that the daily intake of AFA algae is 2 g/day (WHO).

Chloroquine, the known antimalarial drug exerts direct antiviral effect inhibiting pH dependent steps of the replication of several viruses including members of the flavin viruses, retro viruses and corona...
viruses. Moreover, it has immunomodulatory effects suppressing the production/release of tumor necrosis factor α and interleukin 6 which mediate the inflammatory complications of several viral diseases [16].

Recent research showed that vitamin D has an important role in innate immune response against HCV [17]. In addition some studies have shown that vitamin D improves insulin sensitivity (a prediction of liver treatment response) and inhibit HCV replication [18]. Earlier findings [19] specifically in Middle East [20] showed beneficial effect of adding supplement of vitamin D to current standard treatment of HCV as evidenced by improving both early and sustained virological responses. Rhodiol rosea used in the treatment of depression [21,22] has also antiviral effect against Coxackieviruses [23]. It has also hepatoprotective effect.

Milk thistle is a known herbal medicine for treatment of HCV used in conjunction to conventional therapies [24], Ginkgo biloba has hepatoprotective effects against CCl 4-induced oxidative damage may be due to its antioxidant and free radical-scavenging activity [25]. It represses hepatitis C viral replication in HCV viral replication in HCV genotype 1B replicon cell [26]. As all the preceding agents are safe and there is no known non beneficial interaction between them, combination of these agents may be synergetic. This encouraged us to use this combination to treat HCV infection in Egyptian patient. Besides we urged our patients to consume honey, black seeds paste and olive oil in their diet as honey is mentioned in Quran to have beneficial effects to cure patients. Black seeds paste has more beneficial effect than black seed oil as during preparation of oil, there is loss of some beneficial volatile acid. Black seed has immunomodulatory effect [27]. Olive oil contains fat soluble vitamins specially vitamin D and also rich in linoleic acids that has decreasing effect on HCV replication [28].

This work was conducted to determine the efficacy of combination of safe natural product; blue green tablet, vitamin D3, black seeds, olive oil and honey; and chloroquine in treating naive patients with chronic hepatitis C and non-responders to combined IFN/RBV treatment.

**Patients and Methods**

This study enrolled 195 HCV naive patients refusing INF/RBV therapy. Also, patients who failed to achieve sustained virological response to combined Interferon Ribavirin therapy (INF/RBV) whether PEGylated or conventional interferon were included if they stopped the antiviral treatment at least 3 months before recruitment. This study included patients with different stages of fibrosis and patients with compensated cirrhosis. All the patients were treated first from co-infections as schistosomiasis, helicobacter pylori and bacterial infections. Also, nutritional deficiencies were treated before starting from co-infections as schistosomiasis, helicobacter pylori and bacterial infections. In addition patients with less cost.

Blue green® original natural company, Italy; each tablet contains (Rodiola rosea L.) root dry extract (entitled to 1% in salidroside); eleutheroococos (Eleutherococcus senticosus Maxim.) root dry extract (entitled to 5% in saponin); Ginkgo (Ginkgo biloba L.) leaf dry extract (entitled to 24% in ginkgoflavonglycosides and 6% in lactones terpenic); Klamath microalgae (Aphanizomenon flos aquae 50 mg); equiseto (Equisetum arvense L) cauli sterili. The WHO approved that the daily intake of Aphanizomenon flos aquae (AFA) as food supplement is 2 gm/day (WHO). It is recommended from the pharmaceutical company to start with 2 Blue green® tablets/day in empty stomach to prevent gastric upset and can be increases to 4 tablets daily, that was notified to ministry of health in Italy in accordance with art 7 NO 111/1992. In early phase of the study the effect of two tablets daily was not satisfactory so we increased the dose on weight base to 4 tablets daily (equivalent to 100 mg AFA/ 30 kg BW) This dosing was more convenient to the patients with less cost.

Chloroquine was used at dose of 200 mg mg/day for one year for the treatment of HIV (Paton et al. [29]). To reduce the side effect, in this study chloroquine was used in the regimen of treatment and prophylaxis’s of malaria (250 mg/day for 10 days and then every 3 days along the duration of therapy). Vitamin D (1000 IU/day) was used in the recommended daily doses and recommendation reported by Abu Mouch et al. [20], which used Vitamin D supplement to improve SVR in chronic hepatitis C (genotype 1) naive patients treated with peg interferon and ribavirin.

**Subject selection**

Patients included in the study fulfilled the following inclusion criteria: their age 18 years or older, positive Anti-HCV antibody, detectable serum HCV-RNA by quantitative PCR. While patients were excluded if there is evidence of HCC, severe concurrent medical disease such as severe hypertension, heart failure, significant coronary heart disease, poorly controlled diabetes, chronic obstructive pulmonary disease and pregnant women were excluded. All subjects gave written, informed consent before participating in the study.

All patients were subjected to full history taking and thorough clinical examination and the following tests before treatment: abdominal ultrasonography, AST, ALT, serum bilirubin, serum albumin, prothrombin concentration, complete blood count, serum creatinine, viral markers (HCV Ab, HBs Ag), IHA for schistosomiasis and helicobacter antigen in stool were tested for before treatment. Detection of HCV RNA level by PCR quantitative measurements by COBAS Amplipcor 2.0, Roche Molecular Diagnostics, Pleasanton, CA, USA (lower limit of detection of 50 IU/mL) was done before treatment.

All patients had been followed up every three months with physical examination, hematological studies, liver and kidney function tests and abdominal ultra-sonograms. Quantitative HCV-RNA by PCR test was done for each patient after 3, 6, and 12 and 18 months of treatment.

**Statistical analysis**

For the descriptive statistics after having checked the normality of the variables using the Kolmogorov-Smirnov test, the usual central and dispersion methods were used: average, SD, and 95% CI. The statistical significance of differences between the means were evaluated using the paired Student t test in the case of normal distribution of data sets, and using the Kolmogorov-Smirnov test when at least in one of the data sets the normal distribution was excluded. The independent Student t test was used to compare the means of continuous variables and the normal distribution data. Otherwise, the Mann-Whitney U test was used. All statistical analyses were performed using SPSS (statistical package for social science) program version 17 for windows and differences were considered significant at 0.05. Results were given as mean and standard deviation.

**Results**

The Demographic data and present history are shown in table 1. 195 patients were included in this study whose age ranged from 20 years to 76 years. The mean age was 47.8 ± 9.03 years. 132 patients (67.7%) were males while 63 patients were females (32.3%). 57 patients (10.8%) were diabetics, 4 (2.3%) of patients suffered from bilharziasis. 6 patients (3.4%) had minimal ascites. 8 patients had history of previous
Serious side effects and is only effective in approximately 50% of patients. In addition, relapse after SVR may occur [30]. Combination therapy of IFN-α and ribavirin against HCV does not target the virus directly. IFN-α is believed to strengthen the host’s innate antiviral immune response through activation of Janus-activated and tyrosine kinases and through a signal cascade it causes transcription of various genes encoding for proteins that interfere with the virus replicative complex [31]. Regarding ribavirin, the mechanism of action is not well understood; probably it exerts its action through immune-modulatory effects [32]. Drugs target specific viral proteins; especially NS3-4A serine protease and NS5B polymerase are promising. Different peptidomimetic inhibitors, nucleoside analogs, and non-nucleoside analogs are at various stages of development and show high potency against HCV. However, these drugs show a high susceptibility for resistance emergence as detected by in vitro studies. In most cases, one single point mutation is sufficient to achieve tolerance against the drug or worsen cross mutations against one another [33]. These observations indicate that drug resistance is likely to remain a problem, and new drugs targeting viral proteins will not be enough to prevent resistance emergence.

The Mean ALT, AST, Total bilirubin and prothrombin concentration were 54.31 ± 40.26, 56.66 ± 39.97, 1.01 ± 0.73, 77.11 ± 19.09 respectively. The mean creatinine, hemoglobin, total leukocyte count and platelets were 0.93 ± 0.14, 12.52 ± 2.06, 4879.41 ± 1983.08 and 143416.69 ± 72938.82 respectively. The mean HCV RNA was 505538.30 ± 1208201.18 as shown in table 2. Table 3 showed that mean ALT level before treatment was 52.87 ± 36.97 compared to mean ALT level after treatment 38.12 ± 16.47 with highly statistical significant difference (p<0.01). While mean AST level before treatment was 53.46 ± 36.11 compared to mean AST level after treatment 41.61 ± 17.27 with highly statistical significant difference (p<0.01). Mean bilirubin level before treatment was 0.96 ± 0.29 compared to mean bilirubin level after treatment 0.98 ± 0.34 with no statistical significant difference (p>0.05).

In this study, 67/195 patients (34.1%) showed rapid virological response to treatment after 3 months. 82/195 patients (42.1%) showed early virological response to treatment after 6 months (Table 1 and figure 1). Table 2 showed complete response for HBV after 3 months. Two out of 8 (25%) patients who failed to achieve SVR with previous INF/RBV have ETR. There was highly statistical significant association (p value<0.01) between initial HCV RNA level and end of treatment response (347851.32 ± 109928.55). Also, there was statistical significant association (p value<0.05) between initial AST level and end of treatment response (51.49 ± 32.93) as shown in table 5. There is no statistical association (p value>0.05) between end of treatment response and all other studied variables as ALT, bilirubin, albumin levels, INR CBC or age. Other 22 patients showed 2 log decrease of initial HCV RNA after 12 months of treatment but they could not reach negative HCV RNA by PCR at 18 months. There was highly statistical association (p value<0.01) between initial HCV RNA level and 2 log decrease of HCV RNA (1462923.71 ± 205800.24) as shown in table 6. There was no statistical association between all other studied variables and 2 log decrease of initial HCV RNA (Figure 4).

Discussion

The current standard treatment is expensive and also has numerous serious side effects and is only effective in approximately 50% of patients. In addition, relapse after SVR may occur [30].
The lack of vaccine for HCV infections, the high cost of the drugs specially in low-income countries with a high prevalence of HCV, ineffective therapy and the rapid emergence of new drug-resistant viruses have urged a growing need for developing new, more effective chemotherapeutic agents with less side effects for successful HCV treatment. Takehita et al. at the university of Miyazaki in Japan believed that since HCV is localized in the liver and can take 20 years or more to develop into disease, dietary supplement might help slow or stop disease progression [34]. Consumption of AFA has rapid effects on the circulation and function of immune cells in humans. Jensen et al. [35] conducted a randomized, placebo-controlled study to examine the short-term effects of consumption of 1.5 grams of the blue green algae Aphanizomenon flos-aquae (AFA), on the immune system. They found that consumption of an AFA results in rapid changes in immune cell trafficking i.e. generalized mobilization of lymphocytes and monocytes, but not polymorph nucleated cells. This included increases in CD3+, CD4+, and CD8+ T cell subsets and CD19+ B cells with reduction in relative proportions and absolute numbers of Natural Killer (NK) cells. The polymorph nucleated cells showed a mild but significant reduction in phagocytic activity. In vitro, AFA did not induce a direct activation of lymphocytes, as evaluated by tyrosine phosphorylation and proliferative activity. They concluded that AFA increases the immune surveillance without directly stimulating the immune system [15].

Recently there have been many scientifically-controlled studies analyzing the immune enhancing properties of Aphanizomenon flos-aquae (AFA) from Klamath Lake, Oregon. On the basis of the Provisionally Tolerable Daily Intake (PTDI) 1 value established by the WHO, a guidance value of 1 μg microcystin-LR/g AFA algae was issued for AFA algae in the USA and the daily intake of AFA algae is 2 g per person weighted 60 kg [36].

Many patients in Egypt including those who are not eligible for IFN/ribavirin, cannot afford treatment, or fail to respond to IFN, use natural products as alternative treatment for HCV infection. To our knowledge, this is the first study designed to evaluate the effect of combination of natural products in Egyptian patients with chronic HCV. Our results showed that combination of BGA, Vitamin D, black seeds, olive oil and honey and chloroquine affect significantly serum ALT and AST levels. The viral load decreased significantly with treatment. Furthermore, the decrease in viral load was related to the duration of treatment. The virological response was 64.3% after 18 months of treatment.

Baucus and Tanasescu [37] studied the effect of one month treatment of Spirulina (extract of blue green algae) on serum aminotransferases and general state, compared to placebo, in 24 patients with chronic HCV. They found no effect on the level of aminotransferases with improvement in the general status. However, these results may be due to the very short duration of treatment. Yano et al. [38] found that three nutrients β-carotene, vitamin D2, and linoleic acid inhibited HCV RNA replication in a cell culture system and that their combination caused additive and/or synergistic effects on HCV RNA replication.

**Table 5:** Univariate analysis of age, liver functions, CBC and initial HCV RNA in relation to end treatment response.

| Variables        | Mean ± SD | p-value |
|------------------|-----------|---------|
| Age              | 47.81 ± 8.53 | >0.05   |
| ALT              | 52.51 ± 39.96 | >0.05   |
| AST              | 51.49 ± 32.93 | >0.05   |
| Bilirubin        | 0.93 ± 0.32 | >0.05   |
| Serum Albumin    | 3.99 ± 0.34 | >0.05   |
| INR              | 81.25 ± 25.94 | >0.05   |
| HB               | 12.43 ± 1.56 | >0.05   |
| WBCs             | 4600.00 ± 2103.97 | >0.05   |
| Platelets        | 121000.05 ± 74365.56 | >0.05   |
| Initial HCV RNA  | 347851.32 ± 109928.55 | <0.01   |

*Mann Whitney test

**Table 6:** Univariate analysis of age, liver functions, CBC and initial HCV RNA in relation to 2 log decrease of initial HCV RNA.

| Variables        | Mean ± SD | p-value |
|------------------|-----------|---------|
| Age              | 46.32 ± 7.64 | >0.05   |
| ALT              | 50.45 ± 24.04 | >0.05   |
| AST              | 58.80 ± 30.82 | >0.05   |
| Bilirubin        | 1.15 ± 0.49 | >0.05   |
| Serum Albumin    | 3.94 ± 0.34 | >0.05   |
| INR              | 59.57 ± 4.67 | >0.05   |
| HB               | 12.33 ± 3.37 | >0.05   |
| WBCs             | 1462923.71 ± 205800.24 | >0.05   |
| Platelets        | 172750.00 ± 97448.70 | >0.05   |
| Initial HCV RNA  | 1462923.71 ± 205800.24 | >0.05   |

*Mann Whitney test
Conclusion

Combination of safe natural products (Blue green® tablet, vitamin D3, linolenic acid, black seeds and honey) and chloroquine may have a role in treating patients with chronic HCV in combination with recent direct acting antiviral drugs.

References

1. Lauer GM, Walker BD (2001) Hepatitis C virus infection. N Engl J Med 345: 41-52.
2. Pybus OG, Barnes E, Taggart R, Lemey P, Markov PV, et al. (2005) Genetic history of hepatitis C virus in East Asia. J virol 83: 1071-1082.
3. Pybus OG, Charleston MA, Gupta S, Rambaut A, Holmes EC, et al. (2001) The epidemic behavior of the hepatitis C virus. Science 292: 2323-2325.
4. Simmonds P (2004) Genetic diversity of the hepatitis C virus- 15 years on. J Gen Virol 85: 3173-3188.
5. WHO (2002) WHO Traditional Medicine Strategy 2002-2005.
6. Sievert W, Altraif I, Razavi HA, Abdo A, Ahmed EA, et al. (2011) A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver Int 31: 61-80.
7. Camma C, Di Bona D, Schepis F, Heathcoe EJ, Zeuzem S, et al. (2004) Effect of peginterferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data. Hepatology 39: 333-342.
8. Kamal SM, Nasser IA (2008) Hepatitis C genotype 4: what we know and what we don’t yet know. Hepatology 47: 1371-1383.
9. Gilroy DJ, Kaufmann KW, Hall RA, Huang X, Chu FS (2000) Assessing Potential Health Risks from Microcystin Toxins in Blue-Green Algae Dietary Supplements. Environ Health Perspect 108: 435-439.
10. Boyd MR, Gustafson KR, McMahon JB, Shoemaker RH, O’Keefe BR, et al. (1997) Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrob Agents Chemother 41: 1521-1530.
11. Helle F, Wychowski C, Vu-Dac N, Gustafson K, Voisset C, et al. (2006) Cyanovirin-N Inhibits Hepatitis C Virus Entry by Binding to Envelope Protein Glycans. J Biol Chem 281: 25177-25183.
12. Hayashi K, Hayashi T, Kojima I (1996) A natural sulfated polysaccharide, calcium spirulan, isolated from Spirulina platensis: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities. AIDS Res Hum Retroviruses 12: 1463-1471.
13. Aye hunie S, Belay A, Baba TW, Chu FS (1998) Inhibition of HIV-1 replication by an aqueous extract of Spirulina platensis (Arthospira platensis). J Acquir Immune Defic Syndr Hum Retrovirol 21: 722-727.
14. Scheaffer DJ, Krylov VS (2000) Anti-HIV activity of extracts and compounds from algae and cyanobacteria. Ecotoxicol Environ Saf 45: 208-227.
15. Jensen GS, HartAN, Zaske LA, Drapeau C, Gupta N, et al. (2007) Mobilization of human CD34+ CD133+ and CD34+ CD133(-) stem cells in vivo by consumption of an extract from Aphanizomenon flos-aquae–related to modulation of CXCR4 expression by an L-selectin ligand? Cardiovasc Revasc Med 8: 189-202.
16. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R (2003) Effects of an extract from Aphanizomenon flos-aquae–related to modulation of CXCR4 expression by an L-selectin ligand? Cardiovasc Revasc Med 8: 189-202.
17. Adams JS, Hewison M (2010) Update in vitamin D. J Clin Endocrinol Metab 95: 471-478.
18. Bouillon R, Carmellet G, Verlinden L, van Etten E, Verstuyf A, et al. (2008) Vitamin D and human health: lessons from vitamin D receptors null mice. Endocr Rev 29: 726-776.
19. Petta S, Camma C, Scaczone C, Tripodo C, Di Marco V, et al. (2010) Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology 51: 1158-1167.
20. Abu Mouch S, Fireman Z, Jarchovsky J, Assyr N (2010) Vitamin D supplement improve SVR in chronic hepatitis C (genotype 1) naïve patients treated with peg interferon and ribavirin. 45th Annual Meeting of the European Association for the Study of the Liver (EASL 2010). Vienna, Austria.
21. Darbinian Y, Aslanyan G, Armony E, Gabrielyan E, Malmstrom C, et al. (2007) Clinical trial of Rhodolia rosea L. extract SHR-5 in the treatment of mild to moderate depression. Nord J Psychiatry 61: 343-348.
22. Jeong HJ, Ryu YB, Park SJ, Kim JH, Kwon HJ, et al. (2009) Neuraminidase inhibitory activities of flavonols isolated from Rhodiola rosea roots and their in vitro anti-influenza viral activities. Biogeo Med Chem 17: 6816-6823.
23. Wang H, Ding Y, Zhou J, Sun X, Wang S (2009) The in vitro and in vivo antiviral effects of salidroside from Rhodolia rosea L. against coxsackievirus B3. Phytomedicine 16: 146-155.
24. Azzam HS, Goertz C, Fritts M, Jonas WB (2007) Natural products and chronic hepatitis C virus. Liver Int 27: 17-25.
25. Naik SR, Panda VS (2007) Antioxidant and hepatoprotective effects of Ginkgo biloba phytosomes in carbon tetrachloride-induced liver injury in rodents. Liver Int 27: 393-399.
26. Welihang H (2011) Bilobalide, a major ingredient of Ginkgo Biloba leaves, represses hepatitis C viral replication in HCV genotype 1b Replicon cells. AASLD.
27. Tariq M (2008) Nigella sativa seeds: folklore treatment in modern day medicine. Saudi J Gastroenterol 105-106.
28. Irmisch G, Hoepner J, Thome J, Richter J, Fenow A, et al. (2011) Serum fatty acids, antioxidants, and treatment response in hepatitis C infection: greater polyunsaturated fatty acid and antioxidant levels in hepatitis C responders. J Clin Lipidol 5: 288-293.
29. Paton NI, Aboulabh J, Karim F (2002) Hydroxychloroquine, hydroxychloramide, and didanosine as economic treatment for HIV-1. Lancet 359: 1667-1668.
30. Feuerstadt P, Bunim AL, Garcia H, Karltz JJ, Massoumi H, et al. (2010) Effectiveness of Hepatitis C Treatment with Pegylated Interferon and Ribavirin in Urban Minority Patients. Hepatology 51: 1137-1143.
31. Arbuthnot P, Longshaw V, Naidoo T, Weinberg MS (2007) Opportunities for treating chronic hepatitis B and C virus infection using RNA interference. J Viral Hepat 14: 447-459.
32. Lutchanman G, Danewhore S, Song BC, Liang TJ, Hoofnagle JH, et al. (2007) Mutation rate of the hepatitis C virus NS5B in patients undergoing treatment with ribavirin monotherapy. Gastroenterology 132: 1757-1768.
33. De Francesc R, Migliaccio G (2005) Challenges and successes in developing new therapies for hepatitis C. Nature 436: 953-960.
34. Takeshita M, Ishida Y, Akamatsu E, Ohmori Y, Sudoh M, et al. (2009) Proanthocyanidin from blueberry leaves suppresses expression of subgenomic hepatitis C virus RNA. J Biol Chem 284: 21165-21176.
35. Jensen GS, Ginsberg DI, Huerta P, Citton M, Drapeau C (2000) Consumption of an extract from Aphanizomenon flos-aquae–related to modulation of CXCR4 expression by an L-selectin ligand? Cardiovasc Revasc Med 8: 189-202.
36. Eisenbrand G; Senate Commission on Food Safety (SKLM), German Research (2008) Microcystins in algae products used as food supplements. Mol Nutr Food Res 52: 735-736.
37. Mol Nutr Food Res 52: 735-736.
38. Baicus C, Tanasescu C (2002) Chronic viral hepatitis, the treatment with interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology 41-52.
39. Pybus OG, Barnes E, Taggart R, Lemey P, Markov PV, et al. (2005) Genetic history of hepatitis C virus in East Asia. J virol 83: 1071-1082.
40. Pybus OG, Charleston MA, Gupta S, Rambaut A, Holmes EC, et al. (2001) The epidemic behavior of the hepatitis C virus. Science 292: 2323-2325.
41. Simmonds P (2004) Genetic diversity of the hepatitis C virus- 15 years on. J Gen Virol 85: 3173-3188.