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Safety and efficacy of hansenula-derived pegylated-interferon alpha-2a and ribavirin combination in chronic hepatitis C egyptian children

El Naghi *et al.*Customized PEG-IFN-alpha-2a and Ribavirin in HCV-infected children

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**Abstract**

**AIM:** To investigate the safety and efficacy of Hansenula-derived pegylated (PEG) interferon (IFN)-alpha-2a (Reiferon Retard) plus ribavirin customized regimen in treatment-naïve and previously treated (non-responders and relapsers) chronic hepatitis C infected Egyptian children.

**METHODS:** Forty-six children with chronic hepatitis C virus (HCV) infection were selected from three tertiary Pediatric Hepatology centers**.** Clinical and laboratory evaluation were undertaken. Quantitative polymerase chain reaction (PCR) for HCV-RNA was performed before starting treatment, then at 4, 12, 24, 48, 72 wk during treatment and 6 mo after treatment cessation. All patients were assigned to receive a weekly subcutaneous injection of PEG-IFN-alpha-2a plus daily oral ribavirin for 12 wk. Thirty-four patients were treatment-naïve and 12 had a previous treatment trial. Patients were then divided according to PCR results into 2 groups. Group I included patients who continued treatment on a weekly basis (7-day schedule), while group II included patients who continued treatment on a 5-day schedule. Patients from either group who were PCR-negative at week 48, but had at least one PCR-positive test during therapy, were assigned to have an extended treatment course up to 72 wk. The occurrence of adverse effects was assessed during treatment and follow up. The study was registered at www.ClinicalTrials.gov (NCT02027493).

**RESULTS:** Only 11 out of 46 (23.9%) patients showed sustained virological response (SVR), 2 patients were responders at the end of treatment but they were lost to follow up at 6 mo post treatment. Breakthrough was seen in 18 (39.1%) patients, one patient (2.17%) showed relapse and 14 (30.4%) were non-responders. Male gender, short duration of infection, low viral load, mild activity, and mild fibrosis were the factors related to better response. On the other hand, patients with high viral load and absence of fibrosis showed failure of response to treatment. Before treatment, liver transaminases were elevated. After starting treatment, they were normalized in all patients at week 4 and were maintained normal in responders till the end of treatment, while they rose up again significantly in non-responders (*P* = 0.007 and 0.003 at week 24 and 72 respectively). The 5-day schedule did not affect response rate (1/17 had SVR). Treatment duration (whether 48 wk or extended course to 72 wk) gave similar response rates (9/36 *vs* 2/8 respectively; *P* = 0.49). Type of previous treatment (short acting IFN *vs* PEG-IFN) did not affect the response to retreatment. On the other hand, SVR was significantly higher in previous relapsers than in previous non-responders (*P* = 0.039). Regarding safety of the treatment, only mild reversible adverse effects were observed and children tolerated the treatment well.

**CONCLUSION:** Reiferon Retard plus ribavirin combined therapy was safe. Our customized regimen did not influence SVR rates. Further trials on bigger numbers are warranted.

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**Key words**: Children; Chronic hepatitis C; Hansenula polymorpha; Pegylated interferon; Response rate; Ribavirin; Treatment

**Core tip**: Egypt has the highest prevalence of hepatitis C virus (HCV) infection in the world (15%-25%) and the main (90%) genotype is type 4. Prevalence in Egyptian children was found to be 3% in upper Egypt and 9% in lower Egypt. PEG-IFN-alpha-2a or -2b and ribavirin have been used in small numbers of HCV-infected children with SVR being higher in genotypes 2/3 than in genotypes 1/4. A novel 20-kDa PEG-IFN-alpha-2a (Reiferon Retard) derived from *Hansenula polymorpha* expression system have been used in adults with chronic HCV achieving an SVR ranging from 56% to 60.7%, while no studies have been reported in children before.

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**INTRODUCTION**

Hepatitis C virus (HCV) infection is a serious health problem worldwide that establishes a chronic infection in up to 85% of cases[1]. Estimates of prevalence range from less than 1.0% in northern Europe to more than 2.9% in northern Africa[2]. Egypt has the highest prevalence of adult HCV infection in the world (15%-25%) in rural communities[3, 4]and the main (90%) HCV genotype is type 4[5]. Studies of the magnitude of HCV infection in Egyptian children revealed a prevalence of 3% in upper Egypt and 9% in lower Egypt[6].

Blood transfusion was a major risk factor for HCV transmission, but has been virtually eliminated in countries where screening of blood donors is implemented [7]. Vertical transmission of HCV infection is the most common route of acquiring HCV in infants and children[8]. It affects 4%-10% of children born to infected mothers with the highest risk in mothers having a high viral load or co-infected with human immunodeficiency virus[9]. In a large prospective cohort from Egypt, HCV infection was determined in 10% of infants born to infected mothers, 5.47% cleared the virus by 1 year of age and 2.1% cleared the virus by 2-3 years. Persistent infection was detected in 2.43%[10]. HCV infection seems to progress more slowly to fibrosis and cirrhosis in childhood-acquired disease than in adulthood-acquired one[11], even in those who were vertically infected, the infection has been reported as mild[9].

Treatment of chronic HCV aims at slowing disease progression, preventing complications of cirrhosis, reducing the risk of hepatocellular carcinoma, and treating extrahepatic complications of the virus[12].

Currently, standard antiviral treatment for chronic HCV involves once weekly pegylated interferon (PEG-IFN)-alpha injections and daily oral ribavirin. Some reports showed that of adult genotype 4 patients treated with PEG-IFN-alpha and ribavirin, 63% had sustained virological response (SVR)[13, 14].

Treatment individualization has been adopted recently as a therapeutic strategy to improve SVR rate[15]. On treatment virological response appears to be crucial in both tailoring the length of treatment and influencing treatment outcome. It has been reported that early virological response (EVR) at week 12 has a positive predictive value (PPV) of 65%-72% for subsequent SVR while patients with no EVR have no possibility of SVR with a negative predictive value (NPV) of 98%-100%[16, 17]. Whether EVR can be used in children, as in adults, to stop therapy early in patients destined to be non-responders is not clear[18]. Drusano and Preston[19] hypothesized that the longer HCV-RNA remained undetectable after initial clearance, the higher the chance in attaining SVR. Thus it might be expected that extended treatment duration in patients with slow virologic response may improve SVR.

Reiferon retard, a Hansenula-derived novel patented PEG-IFN available in the Egyptian market since 6 years, is a 20 KDa PEG-IFN-alpha-2a. As stated by the manufacturer, *Hansenula polymorpha* represents a stable, robust and safe expression system which has reached the highest productivity of recombinant protein ever described. Reiferon retard has been used in adult Egyptians with safety and efficacy comparable to other pegylated interferons[20-22].

We aimedin the current study at the following points; the first: to investigate the efficacy and safety of Reiferon retard in attaining SVR in treatment-naïve and previously treated (non-responders and relapsers) chronic HCV infected children; the second: to assess the effect of tailoring treatment on SVR [by decreasing the interval between injections (5 d *vs* 7 d) and prolonging duration of therapy (72 wk *vs* 48 wk)] based on the on-treatment virologic response, and the third: to assess predictors of SVR.

**MATERIALS AND METHODS**

***Study population***

This study included 46 children with compensated chronic hepatitis C infection recruited from three Pediatric Hepatology tertiary centers, Pediatric department in Yassin Abdel Ghaffar Charity Center for Liver Disease and Research (YAGCC), Cairo University Pediatric Hospital (CUPH) and Pediatric Hepatology department, National Liver Institute (NLI), between February 2009 and July 2009. The study was completed on August 2011. Diagnosis was based on serological and virological tests; HCV-antibody (Ab) by third generation enzyme linked immunosorbent assay (ELISA) and qualitative and quantitative PCR for HCV-RNA.

Criteria for inclusion were children aged 3-19 years with compensated chronic HCV infection (HCV-RNA positive by PCR for more than 6 mo), whose hemoglobin (Hb) was ≥ 10 g/dL, absolute neutrophilic count (ANC) > 1500/mm3, platelet count > 75000/mm3, and who had normal random blood sugar, serum creatinine, serum ferritin, thyroid function tests and lipid profile and no other associated liver disease [autoimmune hepatitis, Wilson disease, alpha-1 antitrypsin deficiency, hepatitis B virus (HBV) infection]. Liver biopsy was mandatory for enrollment.

Patients with decompensated cirrhosis, any other cause of liver disease associating HCV infection, body mass index (BMI) ≥ 95 percentile, severe psychiatric conditions, uncontrolled seizure disorder, decompensated cardiovascular disease, renal insufficiency, evidence of retinopathy, decompensated thyroid disease, hemoglobinopathy, immunologically mediated diseases or any other chronic illness requiring long term immunosuppressive drugs or previous IFN therapy within one year of enrollment, were excluded from the study. A signed informed consent was obtained from the guardians of all the patients before enrollment in the study. This study was approved by the Research Ethics Committee in the three participating centers.

***Treatment regimens and follow up protocol***

All patients were assigned to receive a weekly subcutaneous injection of PEG-IFN-alpha-2a (Reiferon Retard; Minapharm, Rhein-Biotech, Germany) in a dose of 100 μg/m2 per week plus ribavirin 15 mg/kg daily in two divided doses for a total of 12 wk. Patients were then divided into 2 groups according to HCV-RNA results at week 12.

Group I; patients who continued treatment on a weekly basis (7-day schedule). This group included patients who were HCV-RNA negative at week 12 and those who had < 1 log decrease in HCV-RNA viremia. Group II; patients who continued treatment on a 5-day schedule. This group included patients who had ≥ 1 log decrease in viremia (compared to pre-treatment level) at week 12.

At week 48, patients who were PCR-positive stopped treatment. Patients who were persistently HCV-RNA negative by PCR (at weeks 4, 12, 24 and 48) also stopped treatment and their SVR was checked 6 mo after stopping treatment (SVR 1). Patients who were PCR-negative at week 48 but had at least one PCR-positive test during therapy on week 4, 12, or 24 (delayed response or breakthrough) were assigned to have an extended treatment course of 6 mo duration. PCR was performed at 72 weeks for those patients to detect end of treatment response and those who were HCV-RNA negative, were tested after a further 6 mo for SVR (SVR 2).

All patients were subjected to full history taking and thorough clinical examination before starting treatment, with stress laid on the duration and possible cause of infection, previous trial of antiviral therapy, and psychiatric history in addition to fundus examination. The occurrence of adverse effects was assessed during the treatment and follow up periods.

***Laboratory investigations***

Laboratory investigations, including complete blood count (CBC), albumin, alanine transaminase (ALT) and aspartate transaminase (AST), gamma-glutamyl transpeptidase, alkaline phosphatase, prothrombin time (PT), kidney function tests, alpha-fetoprotein, thyroid function tests (T3, T4, TSH), lipid profile (triglycerides, cholesterol and low and high density lipoproteins), serum autoantibodies (anti-nuclear antibodies, anti-smooth muscle antibodies and liver-kidney microsomal antibodies) and PT were performed for every patient before starting treatment. CBC, ALT and AST were done weekly for the first month, every two weeks for 2 mo and monthly thereafter. PT was performed at the third month and at the end of treatment. Viral markers [HCV-Ab (Innogenetics, Ghent, Belgium), HBV surface antigen, HBV core immunoglobulin (Ig)M and IgG Abs (all from Dia Sorin, Saluggia, Italy)] were performed using ELISA according to the manufacturer instructions. Real-time PCR for HCV-RNA was performed using COBAS® Ampliprep/COBAS® TaqMan®, Roche Molecular Systems, Inc., Branchburg, NJ, 08876 United States (detection limit is 15 IU/mL). According to the viral load, viremia was classified arbitrarily for descriptive purpose into low (≤ 2 × 105 IU/mL) moderate (>2 × 105 – 2 × 106 IU/mL) and high viremia (> 2 × 106 IU/mL). HCV genotyping/subtyping was done by RFLP (restriction fragment length polymorphism) using restriction enzymes *Hae*III, *Rsa*I, *Mva*I and *Hinf*I on PCR-amplified 5'-untranslated region (5'-UTR).

***Dose modification regimen***

The doses of PEG-IFN and ribavirin were modified according to ANC, Hb and platelets. If Hb dropped to < 10 gm/dL, ribavirin dose was to be reduced by 25% and if to < 7.5 gm/dL, erythropoietin was to be administered. If ANC was 500-800/mm3 and/or platelet count < 80000/mm3, IFN dose was to be reduced by 25%. If ANC was 300-500/mm3, IFN dose was to be reduced by 50%. If ANC < 300/mm3 and/ or platelet count ≤ 50000/mm3, IFN dose was skipped and resumed later after the count reached safe levels.

***Definitions of virological response***

Virological responses during therapy were defined as reported by Ghany *et al*[23].

***Liver biopsy and histopathological evaluation***

Liver biopsy was performed for all patients except one who had hemophilia. Hepatic necroinflammatory activity and liver fibrosis were evaluated according to Ishak staging and grading scores[24]. Necroinflammatory activity was classified into mild (score 1-5), moderate (score 6-8), and severe (score 9-18). Fibrosis was classified into mild (stage 1), moderate (stages 2-3), and severe fibrosis or cirrhosis (stages 4-6)[25]. Steatosis was graded semi-quantitatively by determining the percentage of affected hepatocytes and the following scoring system was employed: grade 0: < 5%, grade 1: 5%–33%, grade 2: 34%–66%, grade 3: > 66%[26].

***Statistical analysis***

Descriptive results were expressed as mean ± SD or number (percentage) of individuals with a condition. Statistical significance between groups was tested either by non-parametric test (Mann-Whitney *U* test), Pearson's *χ2* test or Fisher’s exact test. Sensitivity, specificity, PPV and NPV were expressed as percentages. Results were considered significant if *P* ≤ 0.05. Statistical analysis was performed using SPSS software version 13 (SPSS Inc, Chicago, IL, United States).

**RESULTS**

***Patient population characteristics***

Forty-six children were enrolled in the study. They were 33 boys and 13 girls, aged between 4 and 19 years (10.32 ± 3.46 years). Forty-four patients completed the full course of treatment and follow up regimen, while two patients did not show up after completing 48 weeks treatment, so they couldn't be evaluated for SVR. Two patients had glucose-6-phosphate dehydrogenase deficiency, one had hemophilia and one had situs inversus. The demographic and epidemiologic characteristics of the studied population are summarized in Table 1. Blood transfusion was considered a possible risk factor in 34.8% of patients while mother to child transmission was considered a possible one in 17.4% of them. Eighteen patients (39.1%) had an HCV infected family member and most of the patients (43 out of 46) had more than one possible risk factor of infection while in 3 patients we could not reach any possible risk for infection.

The mean of expected duration of infection was 5.29 ± 3.97 years and the mean BMI was 18.20 ± 2.77. Low, moderate and high viremic loads were found in 41.3%, 54.3% and 4.3% of patients respectively. HCV genotype was detected in 38 out of 46 patients. All were genotype 4; 30 (65.2%) were 4a and 8 (17.4%) were 4b. The genotype could not be determined in 8 patients. The majority of patients had mild fibrosis (66.7%) and mild activity (97.8%) in their liver biopsy. Fibrosis was absent in 28.9% of patients while only 4.4% had moderate fibrosis.

***Response to treatment in the group as a whole***

In the group as a whole, only 11 out of 46 (23.9%) showed SVR. We had 14 (30.4%) non-responders where HCV-RNA was detectable throughout treatment. Breakthrough was seen in 18 (39.1%) patients and delayed response in 8 (17%) patients. Relapse occurred in one (2.17%) patient. Two patients had ETR but were lost to follow up and dropped out SVR (Table 2). Figure 1 shows the treatment algorithm according to PCR results for all cases during treatment and follow up periods.

***Predictors of response***

There was no significant statistical difference in response rate of the three centers. Responders were 9 males (9/31 = 29.1%) and 2 females (2/13 = 15.4%). Twelve patients had history of a previous treatment trial, 2 (18.2%) of them achieved SVR while 9 (81.8%) were non-responders. The last one achieved end of treatment response (ETR) but was lost to follow up. The type of previous treatment (short acting IFN *vs* PEG-IFN) did not affect the response to retreatment. On the other hand, SVR was significantly higher in previous relapsers than in previous non-responders (*P* = 0.039). Seven out of the 11 (64%) responders had low viremia. Patients infected with HCV subtypes whether 4a or 4b had similar response rate (4/28 and 1/8 respectively). The majority of patients (44) had mild activity; 11 out of them had SVR (13 achieved ETR). Mild fibrosis was seen in 30 patients; 10 (33%) out of them achieved SVR (12 achieved ETR) while among 13 patients with absent fibrosis, only one (7.7%) achieved SVR. All patients with steatosis (4 patients) did not achieve SVR (Table 3).

***Effect of tailoring treatment according to on- treatment response***

Of the 17 patients who followed the 5-day schedule, one patient (1/17 = 6%) achieved SVR. Extended treatment (72 wk) was given to 8 patients. Whatever the duration of treatment, quarter of cases in each group achieved SVR (Table 3).

***SVR according to rapid virological response and EVR***

Patients who achieved SVR (11/44) had 81.8% rapid virological response (RVR), 90.9% EVR, 100% negative PCR at 24 wk treatment, and 100% ETR. These data are highly significant (Table 4). RVR and EVR had high sensitivity (81.8% and 90.9% respectively) but low specificity (60.6% and 63.6% respectively) in predicting SVR. They had a very good NPV of 90.9% and 95.45% respectively (Table 5).

***Effect of treatment on liver enzymes***

During treatment, ALT and AST normalized in both responder and non-responder groups at week 4 and were maintained normal in responders till the end of treatment, while they rose up again significantly in non-SVR group from week 12 onwards (Figure 2).

***Treatment safety***

Regarding the safety of combined therapy, all side effects were temporary and mild (Table 6). Fever was seen in the first few weeks of treatment in 27 (58.7%) patients and flu-like symptoms appeared in 15 (32.6%) patients. Both anemia and neutropenia were treated by reduction or skipping of doses.

**DISCUSSION**

Since the introduction of IFN, attempts were made to introduce novel IFNs with the aim of increasing therapeutic efficacy, reducing adverse events and/or reducing the cost of therapy.

The current study uses the Hansenula-derived PEG-INF-alpha-2a (a 20 KDa Reiferon Retard) plus ribavirin customized regimen for treatment of chronic HCV infected children.

To date, only 4 Egyptian studies investigating the efficacy and safety profile of the Hansenula-derived PEG-IFN-alpha-2a plus ribavirin for the treatment of adult Egyptian patients with genotype 4 chronic hepatitis C are available. The SVR ranged between 56% and 60.7% following 48 weeks of combination therapy[20, 27-29]. These results are comparable to those obtained using the existing two PEG-IFN-alpha-2a and alpha-2b agents for treatment of genotype 4 in adults[30-33] with the exception of a single report that demonstrated an SVR rate of 33.3% after treatment with PEG-IFN-alpha-2b[34].

In children, Wirth *et al*[35] evaluated the efficacy and safety of PEG-IFN-alpha-2b and ribavirin. Results demonstrated a high SVR of 90% in genotypes 2 and 3, and 53% for children with genotype 1. Another large trial concluded that children with HCV genotype 1 had a 47% response rate with PEG-IFN-alfa-2a/ribavirin[36].

The results of meta-analysis of eight trials[35-42] performed in 2013 by Druyts *et al*[43], indicated that EVR and SVR were each higher for genotypes 2/3 (87% and 89%, respectively) than for genotypes 1/4 (61% and 52%, respectively). The sensitivity analysis comparing PEG-IFN-alfa-2a and PEG-IFN-alfa-2b indicated that these 2 treatments were comparable in terms of efficacy and safety.

In the present study the ETR was 28.2% (2 cases dropped to follow up) and 23.9% showed SVR. Breakthrough was seen in 39.1%. One patient showed relapse and 14 (30.4%) were non-responders.

The lower SVR in genotype 4 infected children in this trial compared to other trials in adults using the same IFN type and in children using other IFN types might be partially explained by the high percentage of previous non-responders (9/12) and relapsers (2/12) included in the study (12/46). Of those 12, only 2 showed SVR (they were the previous relapsers).

Retreatment of children who do not demonstrate an SVR may be beneficial in patients who relapse or show viral breakthrough during treatment, but is not helpful in non-responders[39, 44]. Of our patients, 39.1% had breakthrough and 2.17% showed relapse giving an opportunity for retreatment.

In this study, although the relapse rate is comparable to other studies[39, 43, 45], yet there was a very high rate of breakthrough which may also explain our low response rate as EVR was 24/46 (52%), yet this dropped to an ETR of 13/46 (28.2%) due to the high rate of breakthrough which was 39.1%.

The cause of viral breakthrough is not well understood. Some postulated that it occurs as a result of neutralizing antibodies to IFN, down-regulation of IFN receptors or development of IFN resistance and emergence of quasispecies that are less sensitive to IFN[46]. Also, the overall adherence to ribavirin significantly influences SVR. It is to be noted that only one (2%) patient had ribavirin dose reduction in this study.

The 5-day schedule did not affect response rate. Treatment duration (whether 48 weeks or extended course to 72 wk) gave the same response rate. In the extended treatment group, 4 patients received this extended treatment because of breakthrough (3 at week 24 and one at week 12). All did not benefit from the 72 weeks treatment. On the other hand, 2 out of the 4 patients, who received the extended course because of delayed response, achieved SVR.

In the study of Druyts *et al*[43], only 4% of patients discontinued treatment due to breakthrough.

In our study, 10 out of 11 patients (90.9%) who had achieved SVR had EVR. EVR had a PPV of 45.45% and NPV of 95.45% for SVR. Nine of SVR patients (81.8%) had RVR. RVR had a PPV of 40.9% and NPV of 90.9% for SVR. Thus EVR is slightly better than RVR in negative and positive prediction of SVR. According to Druyts *et al*[43], most of the patients who achieved an EVR (70%) also achieved an SVR (58%). This emphasizes that EVR is crucial and cost-effective in selecting those who can discontinue treatment at week 12 if they remain positive.

According to the present study, factors related to better response were male gender, short duration of infection, low viral load, mild activity, and mild fibrosis. Patients with high viral load and absence of fibrosis showed failure of response to treatment. Also all patients with steatosis (4 patients) failed to achieve SVR. Furthermore, those with previous treatment trials, namely the previous non-responders, showed failure of retreatment with IFN/ribavirin. Novel direct acting antiviral drugs (DAAs), which target specific hepatitis C virus enzymes, showed encouraging results in adults when used alone or in combination with IFN/ribavirin. DAAs have been studied in relapsers, partial responders and null responders to prior PEG-IFN/ribavirin therapy. SVR rates were approximately 85%, 57%, and 31%, respectively[47]. A Japanese study of 10 prior null responders with HCV genotype 1b found 90% SVR using a combination of two DAAs[48]. Such regimens may be of benefit and worth future clinical trials in children to ensure safe and appropriate use of these new agents especially in those with treatment failure to IFN-based therapy.

In other studies[36, 39, 49], predictors of response were early viral response, lower baseline HCV-RNA levels, female sex, non-maternal route of transmission of HCV, absence of steatosis on liver histology, and moderate inflammation on liver biopsy.

Constitutional symptoms are almost universal in children undergoing IFN therapy. Bone marrow suppression induced by IFN constitutes the next most common toxicity after constitutional symptoms, occurring in approximately one third of treatment recipients[35, 36]. In the current study, only mild reversible adverse effects were observed. Fever was seen in the first few weeks of treatment in 58.7% of patients and both the flu-like symptoms and headache appeared in 32.6% of patients. Anemia and neutropenia were found in a rate of 21.7% and 13 % respectively and were treated by reduction or skipping of doses, while none of the patients developed thrombocytopenia. According to a meta-analysis done in 2013[43], neutropenia was the most common hematologic adverse event evaluated (32%), whereas anemia and thrombocytopenia were less frequent (11% and 5%, respectively). Dose reductions due to neutropenia occurred in 38% of patients in the North American study and 12% in the European study respectively[35, 36]. Drug cessation due to neutropenia did not occur in either study. In the North American study[36], there was no significant thrombocytopenia, and in the European study, one patient discontinued therapy at week 42 due to thrombocytopenia (platelet count 45,000 cells/mm3)[35].

The strength of this study is that it is a multicenter one; including 46 children with chronic HCV, using the Hansenula-derived PEG-IFN-alpha-2a (a 20 KDa Reiferon Retard) plus ribavirin, using customized treatment and reporting the response to treatment in children. The limitation is the relatively small sample size.

In conclusion, combined therapy in the form of Reiferon retard plus ribavirin was safe. Children tolerated the treatment well with only mild reversible adverse effects. The end of treatment response was 28.2% and the factors related to better response were male gender, short duration of infection, low viral load, mild activity, and mild fibrosis. Our customized regimen did not influence SVR rate and future clinical trials with novel antiviral drugs may be of benefit to those with treatment failure.

**AKCNOWLEDGEMENTS**

The study was registered at www.ClinicalTrials.gov (NCT02027493).

**COMMENTS**

***Background***

Despite recent success after the introduction of combination therapy with interferon (IFN)-alpha and ribavirin, genotype 4 is considered difficult-to-treat and approximately 60% of patients fail to respond. Resistance to antiviral therapy remains a serious problem in the management of chronic hepatitis C. Establishing novel therapeutic agents, treatment customization, and determining the factors associated with better response rates remain the targets of many researchers.

***Research frontiers***

Reiferon Retard is a novel 20-kDa pegylated (PEG)-IFN-alpha-2a derived from *Hansenula polymorpha* expression system. It has been used in adults with chronic hepatitis C virus achieving an sustained virological response (SVR) ranging from 56% to 60.7%, while no studies have been reported in the pediatric population.

***Innovations and breakthroughs***

The current study is the first one using the novel Hansenula-derived PEG-IFN-alpha-2a in children. Treatment customization regarding duration (72 weeks *vs* extended course of 48 weeks) and IFN injection frequency (5-day schedule *vs* 7-day schedule) demonstrated safety and tolerability in children, yet, it did not improve response rates. This may be, in part, due to the high percentage of previous non-responders included in the study.

***Applications***

The study results suggest that combined therapy in the form of Reiferon Retard plus ribavirin was safe. Children tolerated the treatment well with only mild reversible adverse effects. Treatment duration extension and/or shortening the injection interval did not improve the SVR rates. Male gender, short duration of infection, low viral load, mild activity, and mild fibrosis are associated with favorable response.

***Terminology***

The *Hansenula polymorpha* expression system has been known for its superior characteristics. Due to an increasing number of products and protein candidates derived from this expression system, it has been gaining greater popularity in recent years. *Hansenula polymorpha* represents an absolute mitotic stability, robust and safe expression system which boasts one of the highest productivities ever described for a recombinant protein with maximum purity and high biological activity. In addition, production processes based on *Hansenula polymorpha* technology are very cost effective. The cost effectiveness is strongly related to very short fermentation times and to a significantly reduced number of downstream steps, resulting in a higher purity, with no forms of oxidized interferon being detected.

***Peer review***

Well done study, presented in a detailed fashion. Though it is already known that extending treatment beyond 48 wk achieves little extra benefit, your paper convincingly proves the case for Genotype 4 children (including prior non-responders) which is a not so widely studied sub-group.

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**Figure 1 Treatment algorithm and response to therapy.** At week 4, 24 (52.17%) patients attained rapid virological response (RVR) and 22 (47.82%) had non-RVR. At week 12, five patients (10.86%) had breakthrough while the remaining 19 patients had early virological response (EVR). Those, in addition to 5 patients who turned negative from ones with non-RVR formed a total of 24 (52.17%) patients with EVR. At week 24, those who had breakthrough continued to be polymerase chain reaction (PCR)-positive and 8 patients from those with EVR had breakthrough. The remaining 16 patients in addition to two patients from those with non-EVR turned PCR-negative making a total of 18 (39.13%) patients with negative PCR. At week 48, three of those 18 patients had breakthrough while the remaining 15 patients and one patient from those positive at week 24 in addition to 3 patients who had breakthrough at week 24 (making a total of 19 patients) had negative PCR. Of the 19 negative-PCR patients, 9 had SVR at week 72 and two patients dropped out. The remaining 8 patients had an extended 6-month therapy (3 patients had breakthrough and 5 patients were PCR-negative at their ETR). At week 96, three out of 5 patients with extended course had relapse while the other 2 patients attained SVR making the total SVR 11 out of 44 (25%).

**Figure 2 Alanine aminotransferase and aspartate aminotransferase mean levels during treatment in responders and non-responders.** (A) Alanine aminotransferase (ALT) mean levels kept normal in responders (●) all through the follow up period while in non-responders (■), after being normal, they rose up again to significantly higher levels than in responders from week 12 onwards especially at weeks 24 and 48 (1*P* = 0.007, 0.003 respectively); (B) Aspartate aminotransferase (AST) mean levels kept normal in responders (●) all through the follow up period while in non-responders (■), after being normalized, they significantly rose up again in week 24 (1*P* = 0.007) and once more in week 72.

**Table 1 Demographic, laboratory and histopathological parameters in all patients** ***n* (%)**

|  |  |
| --- | --- |
| **All patients**  **(*n* = 46)** | **Parameter** |
| 10.32 ± 3.46 | Age (yr) |
| 33 (71.7) | Male |
| 5.29 ± 3.97 | Duration of infection (yr) |
| 18.20 ± 2.77 | BMI |
|  | Possible risk of infection |
| 14 (30.4) | Surgery |
| 16 (34.8) | Blood transfusion |
| 5 (10.9) | Tonsillectomy |
| 33 (71.7) | Circumcision |
| 30 (65.2) | Minor procedures1 |
| 8 (17.4) | Vertical transmission |
| 18 (39.1) | Family contact |
| 43 (93.5) | More than one possible risk |
| 3 (6.5) | Unknown risk factor |
| 12.5 ± 1.1 | Hemoglobin (g/dL) |
| 3.13 ± 1.7 | ANC (×10 3/μL) |
| 280.7 ± 82.4 | Platelets (×103 /μL) |
| 4.1 ± 0.37 | Albumin (g/dL) |
| 56.6 ± 55.03 | Alanine transaminase (U/L) |
| 49.2 ± 31.65 | Aspartate transaminase (U/L) |
| 32.1 ± 26.6 | Gamma-glutamyl transpeptidase (U/L) |
| 212.5 ± 96.1 | Alkaline phosphatase (U/L) |
| 12.9 ± 0.62 | Prothrombin time (sec) |
| 3 (15.9) | Hepatomegaly (US) |
| 3 (15.9) | Splenomegaly (US) |
|  | Viremia (IU/mL): |
| 19 (41.3) | Low (15-2×105 IU/mL) |
| 25 (54.3) | Moderate (>2×10 5 - 2×10 6 IU/mL) |
| 2 (4.3) | High (> 2×10 6 IU/mL) |
|  | Genotype: |
| 30 (65.2) | 4a |
| 8 (17.4) | 4b |
| 8 (17.4) | Not determined |
|  | Fibrosis stage |
| 13 (28.9) | Absent |
| 30 (66.7) | Mild |
| 2 (4.4) | Moderate |
|  | Activity grade |
| 44 (97.8) | Mild |
| 1 (2.2) | Moderate |

US: Ultrasound. 1Minor procedures were: Sutures, abscess drainage, ICU hospitalization, endoscopy, ear piercing, tattooing, prolonged hospitalization and dental care.

**Table 2 Response outcome *n* (%)**

|  |  |
| --- | --- |
| **Response type** | ***n* = 46** |
| End of treatment response | 13 (28.2) |
| SVR | 11 (23.9) |
| Dropped out SVR | 2 (4.3) |
| Non-responder | 14 (30.4) |
| Breakthrough | 18 (39.1) |
| Delayed responders ended by breakthrough | 8 (17.4) |
| Breakthrough ended at 48 wk as non-responders | 8 (17.4) |
| Breakthrough ended at 72 wk by relapse | 2 (4.3) |
| Relapse | 1 (2.17) |

Two patients who had end of treatment response (ETR) but were lost to follow up. SVR: Sustained virological response.

**Table 3 Comparison between patients with sustained virological response and non-sustained virological response according to different variables *n* (%)**

|  |  |  |  |
| --- | --- | --- | --- |
| ***P*-value** | **Non-SVR**  **(*n* = 33)** | **SVR**  **(*n* = 11)** | **Parameter** |
|  |  |  | Center: |
| 0.33 | 9 (27.3) | 1 (9) | CUPH |
| 13 (39.4) | 5 (45.5) | YAGCC |
|  | 11 (33.3) | 5 (45.5) | NLI |
| 0.385 | 10.39 ± 3.45 | 9.9 ± 3.76 | Age (yr) |
| 0.34 | 22 (66.7) | 9 (81.8) | Male |
| 0.341 | 5.61 ± 4.02 | 3.77 ± 3.28 | Expected duration of infection (years) |
| 0.546 | 9 (27.3) | 2 (18.2) | Previous treatment trial |
|  |  |  | Previous treatment type2 |
| 1.0 | 8 (88.9) | 2 (100) | Short-acting IFN+RBV |
| 1 (11.1) | 0 (0) | PEG-IFN+RBV |
|  |  |  | Previous response type2 |
| 0.0391 | 7 (77.8) | 0 (0) | Non-responder |
| 2 (22.2) | 2 (100) | Relapser |
|  |  |  | Possible cause of infection |
| 0.709 | 10 (30.3) | 4 (36.4) | Surgery |
| 0.854 | 11 (33.3) | 4 (36.4) | Blood transfusion |
| 0.784 | 4 (12.1) | 1 (9.1) | Tonsillectomy |
| 0.34 | 22 (66.7) | 9 (81.8) | Circumcision |
| 0.709 | 22 (66.7) | 8 (72.7) | Minor procedures |
| 1.0 | 6 (18.2) | 2 (18.2) | Vertical transmission |
| 0.723 | 14 (42.4) | 4 (36.4) | Family contact |
|  |  |  | Injection interval: |
| 0.021 | 16 (48.5) | 1 (9) | 5 d |
| 17 (51.5) | 10 (91) | 7 d |
|  |  |  | Treatment duration: |
| 0.94 | 27 (81.8) | 9 (81.8) | 48w |
| 6 (18.2) | 2 (18.2) | 72w |
|  |  |  | Genotype: |
| 0.89 | 24 (77.4) | 4 (80) | 4a |
| 7 (22.6) | 1 (20) | 4b |
|  |  |  | Viral load: |
| 0.18 | 11 (33.4) | 7 (64) | Low |
| 20 (60.6) | 4 (36) | Moderate |
| 2 (6) | 0 (0) | High |
|  |  |  | Histological Activity: |
| 0.55 | 31 (96.9) | 11 (100) | Mild |
| 1 (3.1) | 0 (0) | Moderate |
|  |  |  | Fibrosis stage: |
| 0.112 | 12 (37.5) | 1 (9.1) | No |
| 18 (56.3) | 10 (90.9) | Mild |
|  | 2 (6.3) | 0 (0) | Moderate |
|  |  |  | Steatosis: |
| 0.46 | 28 (87.5) | 11 (100) | No |
| 3 (9.4) | 0 (0) | Mild |
| 1 (3.1) | 0 (0) | Moderate |

1Significant; 2Percentages were calculated for those with previous treatment trial. SVR: Sustained virological response.

**Table 4 Rapid virological response, early virological response, polymerase chain reaction at week 24, end of treatment response in sustained virological response *vs* non-sustained virological response *n* (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **RVR** | **EVR** | **PCR at week 24** | **ETR** |
| SVR (*n* = 11) | 9 (81.8) | 10 (90.9) | 11 (100) | 11 (100) |
| Non-SVR (*n* = 33) | 13 (39.4) | 12 (36.4) | 0 (0) | 0 (0) |
| *P*-value | 0.0151 | 0.0021 | <0.0011 | <0.0011 |

1Significant. SVR: Sustained virological response; ETR: End of treatment response; PCR: Polymerase chain reaction; EVR: Early virological response.

**Table 5 Predictive value of rapid virological response and early virological response to sustained virological response**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Sensitivity | Specificity | PPV | NPV |
| RVR | 81.8 | 60.6 | 40.9 | 90.9 |
| EVR | 90.9 | 63.6 | 45.45 | 95.45 |

PPV: Positive predictive value; RVR: Rapid virological response; NPV: Negative predictive value; EVR: Early virological response.

**Table 6 Treatment side effects *n* (%)**

|  |  |
| --- | --- |
| **Side effect** | ***n* = 46** |
| Flu like symptoms | 15 (32.6) |
| Headache | 15 (32.6) |
| Fever | 27 (58.7) |
| Injection site reaction | 7 (15.2) |
| Itching | 1 (2.2) |
| Fainting | 1 (2.2) |
| Vomiting | 6 (13) |
| Nervousness | 1 (2.2) |
| Loss of appetite | 10 (21.7) |
| Sleeplessness | 1 (2.2) |
| Rigors | 3 (6.5) |
| Neutropenia | 6 (13) |
| Anemia (Hb 8.5-10 g/dL) | 8 (17.4) |
| Anemia (Hb ≤ 8.5 g/dL) | 2 (4.3) |
| Abdominal pain | 2 (4.3) |
| Arthralgia | 5 (10.9) |
| Diarrhea | 4 (8.7) |