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Can eicosapentaenoic acid maintain the original ribavirin dose or affect the response during the treatment course of chronic hepatitis C virus (HCV) patients?

LIVER

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Original Article

ABSTRACT

Background/Aims: Combination therapy with peginterferon (PEG-IFN) and ribavirin (RBV) has been recommended as a standard therapy for patients with chronic hepatitis C virus (HCV). Our aim was to evaluate the efficacy of eicosapentaenoic acid (EPA) against RBV-associated hemolytic anemia.

Materials and Methods: Two hundred and forty HCV patients included in the study were randomized to either the EPA group (n=120) or non-EPA group (n=120), and they received combination therapy with or without EPA. We compare changes in hemoglobin levels with RBV dose reduction rate in each group as well as treatment response.

Results: Of 120 patients randomized to receive combination therapy with EPA, 15/86 (17.5%) patients required RBV dose reduction, whereas 71/86 (82.5%) patients did not require RBV dose reduction; in the non-EPA group, 22/80 (27.5%) patients required RBV dose reduction and 58/80 (72.5%) patients did not require RBV dose reduction. There was no significant difference between the two groups in the rates of virologic response.

Conclusion: EPA can decrease the rate of RBV dose reduction and RBV-induced hemolysis during the course of combination treatment. Further trials are required to investigate the role of EPA in the current regimens of HCV treatment that include ribavirin.

Keywords: Chronic hepatitis C, ribavirin, eicosapentaenoic acid, anemia, peginterferon

INTRODUCTION

Egypt has a very high prevalence of hepatitis C virus (HCV), which is higher than neighboring as well as other countries, with comparable socioeconomic conditions and hygienic standards; combination therapy with peginterferon (PEG-IFN) and ribavirin (RBV) has been recommended as a standard therapy for patients with chronic hepatitis C. These drugs are administered for either 48 weeks (HCV genotypes 1, 4, 5, and 6) or 24 weeks (HCV genotypes 2 and 3) (1). Unfortunately, this combination therapy is associated with numerous side effects that affect both patients' compliance and response rates (2). Although high RBV doses offer the best chance of a sustained virologic response (SVR), they increase the risk of hemolytic anemia; such effects are, in part, because of oxidative stress induced by RBV. RBV exerts its antiviral activity after intracellular phosphorylation to its monophosphate, diphosphate, and triphosphate that are pharmacologically active forms; this results in the relative deficiency of adenosine triphosphate (ATP) within red blood cells (3). This deficiency of ATP may affect the antioxidant defense mechanisms indirectly, thereby increasing susceptibility to oxidative damage and extravascular hemolysis (4).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which can start chain reactions and can lead to cell damage or death. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by undergoing self-oxidization (5). Although increased reactive oxygen species (ROS) levels in patients with HCV may be beneficial by suppressing HCV replication (6), many studies have demonstrated the beneficial effects of antioxidants on these patients (7).

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The antioxidant fish-derived omega-3s (Omega 300 containing 700 mg EPA) are a group of polyunsaturated fatty acids; its name indicates the position of the first unsaturated bond (double bond) in the hydrocarbon chain that is referred to as n-3, sometimes referred to as omega-3. Omega 3 fatty acids are essential substrates for many of the major regulatory lipids in the body, and because they cannot be synthesized in the body, they are called essential fatty acids. The fish-derived omega-3s family comprises the following fatty acids: α-linolenic acid, stearidonic acid, eicosatetraenoic acid, EPA, docosapentaenoic acid, and docosahexaenoic acid (8).

The fish-derived omega-3s fatty acids play a role in the regulation of cell function by modulating signal transduction via the manipulation of membrane fatty acid composition; in addition, they act as ligands for peroxisome proliferator-activated receptor α (PPARα), a member of the nuclear receptor superfamily, which is associated with cell proliferation and differentiation in hepatocytes and keratinocytes. In addition, omega 3 fatty acids have antithrombotic, immunoregulatory, and anti-inflammatory functions (9). EPA is widely used to treat hyperlipidemia and atherosclerosis because it readily assimilates into erythrocyte membrane phospholipids and correlates positively with erythrocyte deformability. In an uncontrolled pilot study, patients who were administered EPA after the development of anemia showed significant increases in mean hemoglobin (Hb) values, and these prospective study data indicate that EPA can suppress RBV-induced anemia (10).

This study aims to evaluate the efficacy of EPA against RBV-associated hemolytic anemia during HCV combination treatment course and response to treatment.

MATERIALS AND METHODS

This study was conducted in Tropical Medicine and Gastroenterology Department, Faculty of Medicine, Sohag University and Sohag Centre of Gastroenterology, from May 2014 to April 2015 after approval by the local ethics committee. Two hundred and forty patients with chronic HCV infection were included in this study (71 females and 169 males) after obtaining their informed written consent; patients were selected randomly from patients visiting HCV treatment unit for pretreatment evaluation.

All patients met the following inclusion criteria: chronic infection with HCV according to seropositivity for anti-HCV antibodies, age of at least 18 years, Hb level of at least 12 g/dL, compensated liver disease, and no hepatocellular carcinoma.

Exclusion criteria were as follows: previously treated patients; obese patients [body mass index (BMI) of >30]; HBV coinfection; decompensated liver disease; liver cirrhosis (pathologically proved); autoimmune disease; clinically significant cardiovascular, metabolic, renal, hematologic, neurologic, or psychiatric disease; hematologic, neurologic, or psychiatric disease.

All patients were subjected to the following:

- Careful history-taking and thorough clinical examination with calculation of BMI.
- The following laboratory tests: complete blood count, liver function tests, coagulation profile, kidney function tests, thyroid function tests, serologic markers for hepatitis B virus, antinuclear antibodies, quantitative polymerase chain reaction (PCR) for HCV RNA (real-time technique), fasting blood glucose level, and glycosylated Hb A1C if fasting blood glucose is elevated
- Electrocardiography (ECG)
- Fundus examination of the eyes
- Pelvi-abdominal ultrasonography

The patients were divided into two groups

I-Test group (EPA group)

- This group comprises 120 patients (35 females and 85 males). They received subcutaneous injection of pegylated interferon alpha 2a at a dose of 180 µg weekly with RBV therapy at a dose of 15 mg/kg daily divided in three doses orally administered together with the antioxidant fish-derived omega-3s regimen administered twice daily via the oral route.

II-Control Group (non-EPA group)

- This group comprises 120 patients (36 females and 84 males). They received the same interferon/RBV therapy alone.

Patients follow-up

1- After 4 weeks: Complete blood count and liver function tests were performed for all patients.

2- After 12 weeks: Complete blood count and liver function tests were performed for all patients. A quantitative PCR for HCV RNA was also performed for all patients, according to which the patients will be divided into three categories as follows:
   - Null responders (patients who failed to achieve a decline by at least 2 log in their viral load) and for whom treatment was stopped.
   - Partial early responders (pEVR; patients who managed to achieve the 2-log decline in their viral load but still their PCR test was positive).
   - Complete early responders (cEVR; patients with negative PCR).

The last two categories will continue treatment regimen prescribed for them according to their group.

3- After 24 weeks: the following laboratory tests were performed for all patients: complete blood count and liver function tests as well as qualitative PCR for HCV RNA, according to which they were divided into the following two groups:
   - Positive PCR: treatment was stopped.
   - Negative PCR: treatment was continued.

4- After 48 weeks: the following laboratory tests were performed for all patients: liver function tests and complete blood count as well as qualitative PCR for HCV RNA. For

anemia, the daily RBV dose was reduced by 200 mg when Haemoglobin (Hb) level decreased below 10 g/dL, acute decrease and remains of Hb concentrations >3g/dL from baseline, or clinical symptoms of anemia associated with a decrease of Hb >2g/dL from the start of treatment.

The course of treatment was stopped in the following situations:

- Partial or null responders: Patients who did not achieve a reduction in HCV RNA level by week 12 or 24 of treatment.
  Marrow suppression: in particular, leucopenia, neutrophil counts less than 500 cells/µL, thrombocytopenia, platelet count less than 75,000/mm^3.
- Emotional effect: major uncontrolled depressive illness during therapy, an indication for discontinuation.
- Visual disorder (rarely retinal hemorrhages, particularly in diabetic and hypertensive patients.
- Seizures, hearing loss, pancreatitis, and interstitial pneumonitis.
- Hemolytic anemia: the RBV drug was permanently discontinued at levels of Hb less than 8.5 gm/dL.

Ethical considerations

The protocol was approved by the local ethics committee in our faculty, and all patients gave their written informed consent before being included in the study.

Statistical analysis

Data were checked, entered, and analyzed using the SPSS Statistical Package, Version 15.0, software (SPSS Inc.; Chicago, IL., USA). Data were expressed as arithmetic mean (X)±standard deviation (SD) for quantitative variable, number, and percentage for qualitative one. Chi-square (X^2) and t test were used when appropriate. McNemar's test of significance was used in cross-over comparisons, and a p value of less than 0.05 was considered to be significant and that of less than 0.001 was considered to be highly significant.

RESULTS

Baseline characteristics and values are summarized in Table 1, and there were no significant differences between the two groups with regard to any of these parameters.

Eicosapentaenoic acid (EPA) group

Of 120 patients randomized to receive combination therapy with EPA, 34/120 (28.4%) patients withdrew from the study before completing the course of treatment and 86/120 (71.6%) patients completed the course of treatment; 15/86 (17.5%) patients required RBV dose reduction, whereas 71/86 (82.5%) patients needed no RBV dose reduction.

Non-eicosapentaenoic acid (EPA) group

Of 120 patients randomized to receive combination therapy without EPA, 40/120 (33.4%) patients withdrew from the study before completing the course of treatment and 80/120 (66.6%) patients completed the course of treatment; 22/80 (27.5%) patients required RBV dose reduction, whereas 58/80 (72.5%) patients needed no RBV dose reduction.

There was a significant difference between both groups regarding the development of anemia throughout the treatment period, as shown in Table 2 and Figure 1; in addition, there was a significant difference between both groups regarding the requirement of RBV dose reduction for anemia, as shown in Table 2. WBC count was significantly lower in the control group during the course of treatment (Figure 2).

Table 1. Baseline characteristics and values of the patients

	Test group (n=120)	Control group (n=120)	p
Male/Female	85/35	84/36	0.88
Age, years	35 (20–57)	33.3 (19–55)	0.06
ALT level (IU/mL)	61.9 (20–160)	68.7 (16–293)	0.17
AST (IU/mL)	50.2 (17–123)	49.7 (14–191)	0.87
Alkaline phosphatase (IU/mL)	89.3 (42–189)	85.6 (52–200)	0.5
Bilirubin (mg/dL)	0.6 (0.5–2)	0.7 (0.4–2.5)	0.1
Albumin (g/dL)	4.5 (3.6–4.7)	4.4 (3.8–4.9)	0.5
Hb (g/dL)	13.9 (11–16.7)	14.2 (11.5–16)	0.88
WBC (cells/µL)	6.1 (3.2–11.4)	6.5 (3.3–12.2)	0.16
PLT (cells/µL)	177.8 (100–309)	180.7 (100–275)	0.69

Values are given as median (minimum–maximum).
ALT: alanine aminotransferase; AST: aspartate aminotransferase; Hb: hemoglobin; WBC: white blood cells; PLT: platelets

Table 2. Hemoglobin changes and the percentage of significant anemia (Hb<10 gm/dL) throughout the follow-up period in both groups

Items	Hb (gm/dL)	Week 12	Week 24	Week 48	Signif. Anemia need RBV dose reduction (Hb<10 gm/dL)
Test group	X±SD	12.4±1.2	11.8±1.9	11.7±1.9	15/86 (17.5%)
Control group	X±SD	11.8±1.9	11.1±1.34	11.0±1.3	22/80 (27.5%)
p		0.03	0.05	0.012	0.049

Hb: hemoglobin

Table 3. Frequencies of anemia-related symptoms in both groups

Side Effect	EPA group n=120 (%)	Non-EPA group n=120 (%)	p
Dyspnea	20 (16.7%)	35 (29.2%)	0.01
Fatigue	31 (25.8%)	42 (35%)	0.06
Dizziness	22 (18.3%)	35 (29.2%)	0.02

EPA: eicosapentaenoic acid

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Table 4. Serum bilirubin changes during the course of treatment in both groups

Bilirubin (mg/dL) (mainly indirect)	Test group (n=120)	Control group (n=120)	p
4 weeks	0.9±0.2	1.6±0.4	<0.001
12 weeks	1.3±0.3	2.1±0.5	<0.001
24 weeks	1.5±0.2	2.7±1	<0.001
48 weeks	1.9±0.5	2.7±0.6	<0.001

Data are expressed as Mean±SD

Table 5. The rates of virologic response throughout the treatment period

Virologic response	Test group	Control group	p
cEVR (complete early virologic response at 12 weeks	95/120 (79.16%)	92/120 (76.6%)	0.64
pEVR (partial early virologic response at 12 weeks	25/120 (20.84%)	28/120 (23.4%)	0.64
24 weeks	86/120 (71.6%)	80/120 (66.6%)	0.45
End treatment response (48 weeks)	60/86 (69.1%)	52/80 (65%)	0.51
Sustained virologic response (SVR)	49/86 (57%)	41/80(51%)	0.7

Data are expressed as (n, %)

Table 6. The percentage of discontinuation of treatment in both groups

	Group I study group n=120 (n,%)	Group II control group n=120 (n,%)	p
Total discontinuation	34/120 (28.4%)	40/120 (33.4%)	0.55
Null responders	27 (22.5%)	33 (27.5%)	0.47
Anemia (Hb<8.5 gm/dL)	3 (2.5%)	4 (3.33%)	0.85
Neutropenia	4 (3.33%)	3 (2.5%)	0.85

Hb: hemoglobin

There were no significant differences between the two groups with regard to platelet count, as shown in Figure 3. Table 3 showed that anemia-related symptoms during the course of treatment were higher among the patients in the non-EPA group.

Table 4 showed significantly lower levels of bilirubin (mainly indirect) in the test group during the course of treatment. There was no significant difference between the two groups with regard to the rates of virologic response throughout the treatment period (Table 5). There was no significant difference between both groups regarding the percentage of patients who discontinued the treatment, as shown in Table 6.

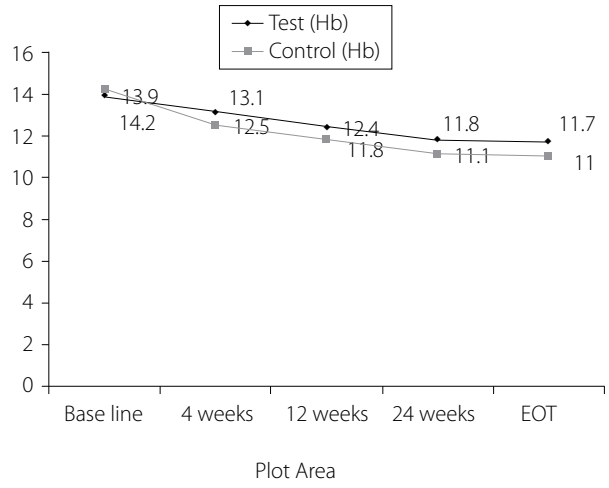

Figure 1. Linear representation of hemoglobin concentrations throughout the treatment period in test and control groups.
Hb: hemoglobin; EOT: end of therapy

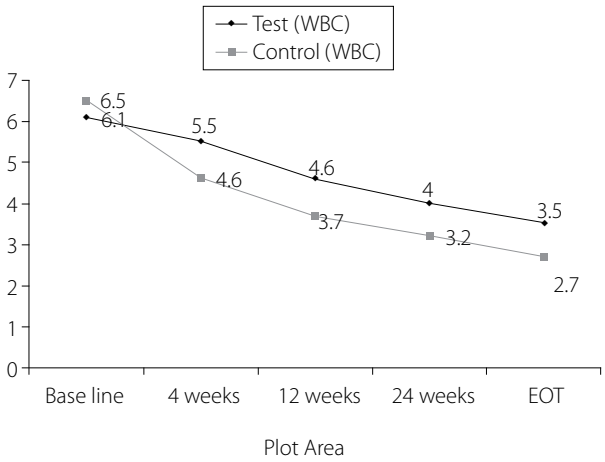

Figure 2. Linear representation of WBC counts throughout the treatment period in test and control groups.
WBC: white blood cell; EOT: end of therapy

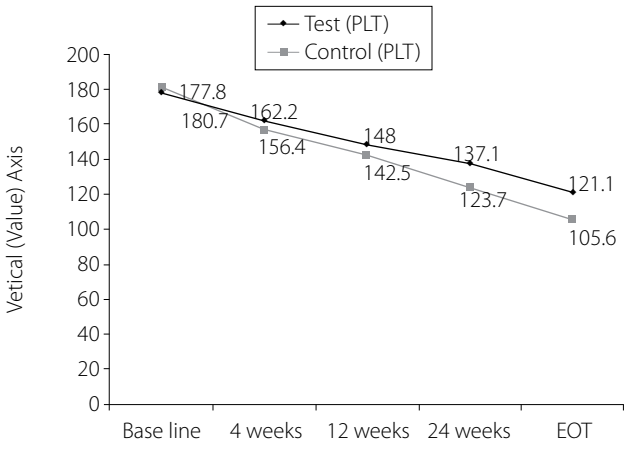

Figure 3. Linear representation of platelet counts throughout the treatment period in both test and control groups.
PLT: platelets; EOT: end of therapy

DISCUSSION

The results of this study demonstrated that with regard to EPA treatment, anemic HCV-infected patients who underwent combination therapy more often could avoid RBV dose reduction during the course of therapy. Many previous studies reported that combination therapy with PEG-IFN and RBV for 24–48 weeks was effective in treating chronic hepatitis C (11). The most frequent toxic side effect of RBV is reversible hemolytic anemia, which requires some patients to reduce or discontinue combination therapy; the mechanism underlying hemolytic anemia involves the accumulation of RBV triphosphate in erythrocytes (12).

The effect of RBV accumulation on cellular respiration reduces the half-life of erythrocytes via extra vascular hemolysis; EPA, the principle fatty acid in fish oils, has various pharmacologic actions such as an increase in the deformability of erythrocytes (4). EPA has been shown to improve erythrocyte deformability by incorporation in erythrocyte membrane phospholipid (13). Although Hino et al. (14) demonstrated a decrease in EPA concentration in erythrocyte membrane phospholipid in HCV patients with IFN and RBV therapies, it is considered that EPA supplementation may be useful for preventing RBV-induced anemia.

This study was designed to concentrate on the direct effect of the use of omega 300 (EPA) with combination therapy (pegylated interferon and RBV) on virologic response, providing little interest to some RBV-associated complications (hemolytic anemia) that are supposed to be affected by Omega 300 (EPA).

Omega 3 has been widely used in the prevention of cardiovascular disease, and at least 1 gm should be used daily (15); the dose may be increased up to 4 gm, but nausea and GIT upset may limit the increase in the dose. The omega 300 (EPA) used in this study is one of the most widely used antioxidant fish-derived omega-3s in the commercially available combinations in the Egyptian drug market, and its dose is also determined according to average available doses in those combinations. These combinations are sold as dietary supplements for patients with chronic as well as those with acute liver disease; several of those combinations enjoy the support of clinicians as well as are preferred by patients. It was important to take, more or less, a sample of the ingredients in these combinations at their average dose available in these combinations. We chose the most common ingredient, i.e., the fish-derived omega-3s (Omega 300 containing 700 mg EPA), which is administered through the oral route twice daily. In our study, we used a daily dose of 700 mg EPA twice (1400 mg) daily; this dose was higher than that used by Takakai et al. (16) who used a daily dose of 900 mg in a similar study.

In our study, we evaluated hematological, biochemical, and virological parameters measured at 4, 12, 24, and 48 weeks. There are several complications of interferon/RBV therapy. Of them, we studied the most important complications, namely hematological complications. The mean Hb concentration was nonsignificantly different in the two groups before treatment. It became significantly lower in the control group throughout the follow-up period. The rate of decline of Hb concentration was significantly higher in the control group. The percent of change was also significantly higher in the control group. Our results are supported by the results of DeFranceschi et al. (4) who demonstrated the central role of oxidative stress in the RBV-induced hemolysis.

Our results also agree with Hino et al. (14) who concluded that fish-derived omega-3s supplementation enabled stopping the premature destruction of RBCs by maintaining the ecosapentaenoic acid level in their cell membranes from decreasing because of interferon therapy. Hino et al. (14) also ascertained that the oxidative damage in erythrocyte membrane plays an important role in RBV-induced anemia.

Because the RBV concentrations remained stable after 4–8 weeks of treatment, it is considered that RBV does not contribute to the antiviral effect during 4 weeks after the beginning of combination treatment (17). However, Arase et al. (18) demonstrated that a higher concentration contributes to SVR and that to increase the RBV concentration, it is important to maintain a RBV dose in the early phase after beginning the combination treatment.

Mean WBC count was also nonsignificantly different in two groups before treatment. It became lower in the control group throughout the follow-up period, and this result was highly significant. The rate of decline and percent of change were significantly higher in the control group, but we did not find an explanation to this finding; we recommend that it is a potential research topic in future studies. Mean platelet count was nonsignificantly different in the two groups before treatment and throughout the treatment period. However, the rate of decline was significantly higher in the control group. Our results are comparable to those of Rustgi et al. (19) who found that platelet count decreases over a longer period than the other hematological parameters.

In our study, serum bilirubin was studied. The bilirubin became highly significantly in the control group starting from week 4 to the end of therapy. This can easily be explained on the background that EPA prevents RBV-induced hemolysis. The increase in indirect bilirubin was also associated with a decline in Hb concentration in both groups. This emphasizes that hyperbilirubinemia is because of hemolysis. The rate of increase as well as the percent of change of bilirubin level was significantly higher in the control group.

In the EPA group, of 120 patients randomized to receive combination therapy with EPA, 34/120 (28.4%) patients withdrew from the study before completing the course of treatment and

86/120 (71.6%) patients completed the course of treatment; 15/86 (17.5%) patients required RBV dose reduction, whereas 71/86 (82.5%) patients needed no RBV dose reduction.

In Non-EPA group, of 120 patients randomized to receive combination therapy without EPA, 40/120 (33.4%) patients withdrew from the study before completing the course of treatment and 80/120 (66.6%) patients completed the course of treatment; 22/80 (27.5%) patients required RBV dose reduction, whereas 58/80 (72.5%) patients needed no RBV dose reduction.

We found no significant difference between the two groups with regard to the rate of virologic responses; RVR, EVR, 24 weeks, EOT and SVR but the symptoms of anemia among EPA group were significantly less than those in the other group; this may be because the development of anemia throughout the treatment period was less among the patients in the EPA group, thereby leading to better adherence of patients to treatment. We have followed good patient selection criteria during our study according to our national protocol because we excluded cirrhotics on the basis of liver biopsy, obese patients, patients with HBV coinfection, null responders and relapsers lead to a good SVR result: our results are in agreement with those of the study by Al Ashgar et al. (20) who reported an SVR of 50.7%.

The limitation of our study is that we did not include patients with cirrhosis or those who received prior treatment because we were guided by the protocol of our National Committee for Control of Viral Hepatitis in treatment centers.

In conclusion, based on the abovementioned results, we can conclude that the use of antioxidant fish-derived omega-3s (Omega 300 containing 700 mg EPA) can prevent serious hematological complications of INF/RBV combination therapy such as treatment-associated anemia and can decrease the rate of RBV dose reduction during the course of combination therapy but cannot affect the treatment response. We recommend this medication for every patient on regimens including RBV for treating patients with HCV to decrease the symptoms of anemia for better compliance and treatment adherence.

These results can improve the quality of life and adherence to therapy and can justify the use of this antioxidant fish-derived omega-3s as an adjunctive therapy with pegylated interferon and RBV. Further large-scale, double-blind, randomized controlled trials are required to investigate the role of EPA in the current regimens of HCV treatment with direct acting antiviral drugs that include RBV.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Sohag University.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - K.M., A.Z., M.M.; Design - K.M., A.Z., M.M.; Supervision - K.M., A.Z., M.M.; Resource - K.M., A.Z., M.M.; Materials - K.M., A.Z., M.M.; Data Collection and/or Processing - K.M., A.Z., M.M.; Analysis and/or Interpretation - K.M., A.Z., M.M.; Literature Search - K.M., A.Z., M.M.; Writing - K.M., A.Z.; Critical Reviews - K.M., A.Z.

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REFERENCES

1. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347: 975-82. [CrossRef]
2. Keating GM, Curran MP. Peginterferon-alpha-2a (40kD) plus ribavirin: a review of its use in the management of chronic hepatitis C. Drugs 2003; 63: 701-30. [CrossRef]
3. Patterson JL, Fernandez-Larsson R. Molecular mechanisms of action of ribavirin. Rev Infect Dis 1990; 12: 1132-46. [CrossRef]
4. De Franceschi L, Fattovich G, Turrini F, et al. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. Hepatology 2000; 31: 997-1004. [CrossRef]
5. Sies H. Oxidative stress: Oxidants and antioxidants. Exp Physiol 1997; 82: 291-5. [CrossRef]
6. Choi J, Lee KJ, Zheng Y, Yamaga AK, Lai MM, Ou JH. Reactive oxygen species suppress hepatitis C virus RNA replication in human hepatoma cells. Hepatology 2004; 39: 81-9. [CrossRef]
7. Singal AK, Jampana SC, Weinman SA. Antioxidant as therapeutic agents for liver disease. Liv Int 2011; Nov; 31: 1432-48. [CrossRef]
8. Calder PC. Polyunsaturated fatty acids and cytokine profiles: a clue to the changing prevalence of atopy? Clin Exp Allergy 2003; 33: 412-5. [CrossRef]
9. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 2000; 192: 1197-204. [CrossRef]
10. Ide T, Okamura T, Kumashiro R, et al. A pilot study of eicosapentaenoic acid therapy for ribavirin-related anemia in patients with chronic hepatitis C. Int J Mol Med 2003; 11: 729-32. [CrossRef]
11. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001; 345: 41-52. [CrossRef]
12. Takaki S, Tsubota A, Hosaka T, et al. Factors contributing to ribavirin dose reduction due to anemia during interferon alfa2b and ribavirin combination therapy for chronic hepatitis C. J Gastroenterol 2004; 39: 668-73. [CrossRef]
13. Mori TA, Watts GF, Burke V, Hilme E, Puddey IB, Beilin LJ. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. Circulation 2000; 102: 1264-9. [CrossRef]
14. Hino K, Murakami Y, Nagai A, et al. Alpha tocopherol [corrected] and ascorbic acid [corrected] attenuates the ribavirin [corrected] induced decrease in ecosapentaenoic acid in erythrocyte membrane in chronic hepatitis C patients. J Gastroentrol Hepatol 2006; 21: 1269-75. [CrossRef]
15. Roosha K, Parloop B.Omega-3 polyunsaturated fatty acid and cardiovascular disease: A Review. Gujarat Medical Journal 2010; 7: 66-70.

16. Takaki S, Kawakami Y, Imamura M, et al. Eicosapentaenoic acid could permit maintenance of the original ribavirin dose in chronic hepatitis C virus patients during the first 12 weeks of combination therapy with pegylated interferon α and ribavirin. Intervirology 2007; 50: 439-46. **[CrossRef]**
17. Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon a2b plus ribavirin for 48 weeks or for 24 weeks versus interferon a2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998; 352: 1426-32. **[CrossRef]**
18. Arase Y, Ikeda K, Tsubota A, et al. Significance of serum ribavirin concentration in combination therapy of interferon and ribavirin for chronic hepatitis C. Intervirology 2005; 48: 138-44. **[CrossRef]**
19. Rustgi VK, Lee P, Finnegan S, et al. Safety and efficacy of recombinant IL-11 (oprelvekin) in combination with interferon/ribavirin in hepatitis C patients with thrombocytopenia. Hepatology 2002; 36(4 Pt 2): 361A.
20. Al Ashgar H, Helmy A, Khan MQ, et al. Predictors of sustained virological response to a 48-week course of pegylated interferon alfa-2a and ribavirin in patients infected with hepatitis C virus genotype 4. Ann Saudi Med 2009; 29: 4-14. **[CrossRef]**