Effect of L-carnitine and conjugated linoleic acid supplements on haemoglobin levels and haptoglobin genotype in chronic kidney disease

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Abstract

Objective: To investigate the efficacy of L-carnitine (LC) and conjugated linoleic acid (CLA) supplements on haemoglobin levels and inflammatory markers in chronic kidney disease (CKD) patients with different haptoglobin (HP) genotypes.

Methods: This clinical trial study was conducted at Imam Khomeini Hospital, Ardabil, and Labbafinejad Hospital, Tehran, Iran, from March 2014 to March 2015, and comprised male patients with CKD and anaemia. Anthropometric factors were recorded and demographic data was collected using general questionnaires. LC (1 g/day) and CLA (2.4 g/day) supplements were given to the patients for a month. Blood samples were taken to measure haematological and inflammatory markers at the beginning and end of the study. Haptoglobin genotypes were determined using polymerase chain reaction (PCR). SPSS 21 was used for data analysis.

Results: Among the 40 patients in the study, HP2-2 genotype was the most prevalent genotype (62.5%). The level of haemoglobin was significantly increased in the patients at the end of the study (p< 0.05). No significant changes were found in the weight, body mass index and serum levels of Interleukin-6, high-sensitivity C-reactive protein, ferritin, total iron-binding capacity and iron (p>0.05 each).

Conclusion: Regular diet supplementation with LC plus CLA can improve haemoglobin levels in CKD patients with anaemia.

Keywords: Haptoglobin genotype, CKD, Anaemia, CLA, L-carnitine. (JPMA 69: 343; 2019)

Introduction

Chronic kidney disease (CKD) is a worldwide public health threat which is a cause of considerable increase in cardiovascular morbidity and premature mortality. The prevalence of CKD stages 3-5 in Iran is 14.9%.1 Anaemia is a common complication of CKD2 which may be due to lack of erythropoietin, low folic acid, vitamin B12 and iron levels.3 It has been shown that correction of anaemia is associated with improved outcomes in CKD patients.4 In patients with CKD, anaemia can be treated using recombinant human erythropoietin, but some of these patients develop resistance to erythropoietin.5 In CKD, chronic inflammation plays an important role in the disease process.6 It has been reported that the resistance to erythropoietin treatment is one of the important outcomes of chronic inflammation in CKD.7 Several proteins such as haptoglobin (HP) are produced from hepatocytes during chronic inflammation in CKD. HP binds to haemoglobin (Hb) to prevent renal clearance of Hb and renal damage caused by free Hb.8 HP-Hb complex modulate inflammation by the inhibition of prostaglandin synthesis.9 HP is encoded by 2 distinct alleles of HP1 and HP2, and these two alleles produce 3 phenotypes: HP1-1, HP1-2 and HP2-2.10 The frequency of HP alleles varies among different populations. In addition, HP levels may be dependent on HP phenotype, so that those with the HP1-1 phenotype have the highest HP serum concentrations.11

HP participates in Hb and iron metabolism. It may affect anaemia and Hb levels by controlling the inflammation process. It has been shown that different HP phenotypes can cause various responses in the host against inflammation.12,13 The antioxidant effects of nutrient supplement such as conjugated linoleic acid (CLA) and L-carnitine (LC) have been investigated separately in CKD.14,15 CKD patients undergoing haemodialysis usually suffer from LC deficiency.16 LC has an inhibitory property on inflammatory markers, lipid peroxidation and apoptosis pathways.17 Furthermore, LC supplements may increase haematocrit (HCT) levels and decrease erythropoietin requirement.18,19

CLA is another diet supplement that is important in human nutrition due to its health benefits such as being anti-inflammatory, anti-obesity, anti-cancer, anti-atherosclerosis and anti-diabetic.20 A number of studies showed that CLA prevents the secretion of inflammatory cytokines and modulates the host inflammatory response.21
The current study was planned to evaluate the efficacy of CLA and LC combination on Hb level and inflammatory factors in CKD patients with different haptoglobin genotype.

Materials and Methods
This clinical trial study was conducted at Imam Khomeini Hospital, Ardabil, and Labbafinejad Hospital, Tehran, Iran, from March 2014 to March 2015. During one-year period of time, based on inclusion criteria, we selected 50 CKD patients with anaemia referring to these two centres. Ten participants of them were excluded due to non-cooperation and abuse of supplements. Finally, 40 male CKD patients with anaemia from 18-60 years old were recruited. CKD was defined as glomerular filtration rate (GFR) = 15-90 mL/min and anaemia was defined as Hb less than 13g/dl.

The sample size was determined according to the formula:\[ N = \frac{\left( Z_\alpha + Z_\beta \right)^2}{C^2} + 3, \quad C = 0.5 \times \ln\left(\frac{1+r}{1-r}\right) \]
In the formula, \( N \) = total sample size, \( C \) = expected correlation coefficient, \( Z_\alpha = \) the probability of committing a type I error, \( Z_\beta = \) the probability of committing a type II error, \( r = \) the correlation coefficient, ln = natural logarithm.

CKD patients suffering from other chronic diseases such as cardiovascular diseases, thyroid disease, malignancies, diabetes and those who took iron supplements were excluded.

The study was approved by the research ethics committee of Ardabil University of Medical Sciences (Ethical code: Arums.REC.93.65) and recorded in the Iranian Registry of Clinical Trials identification code of IRCT201503055144N7. Written informed consent was also obtained from each participant before the initiation of the study. After getting the consent, general information, anthropometric factors (height and weight) and fasting blood samples, both supplements of LC (1gm per day) and CLA (2.4 gm per day) were given to subjects by oral administration for one month. The possible risks and complications of the pills were explained to the patients.\(^{23}\) The patients were called every week for follow-up and it was ensured that the participants had consumed the supplements. The patients' compliance with the consumption of the pills was evaluated by the remaining pills at the end of the 4th week. The patient's weight and height were recorded and their body mass index (BMI) was calculated and categorised as per defined standards.\(^{24}\) The individuals' fasting weights were measured with an accuracy of 0.5 kg. Each time at certain stages, accuracy and precision of the Seca scales were calibrated using a standard 5kg weight. In the beginning and at the end of the study, after 10-12 hours of overnight fasting, 5ml venous blood sample was taken from all patients. The Hb and HCT were measured using cell counter. Serum was separated from the whole blood and kept frozen at -80°C until experimental analysis. The serum levels of iron and total iron-binding capacity (TIBC) were measured using the photometric method. Serum levels of Interleukin 6 (IL-6) were measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer’s instructions (ZellBio GmbH, Ulm, Germany).

Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes using CinnaPure® DNA extraction Kit (SinaClon, Tehran, Iran). The primers A (5’-GAGGGGAGCTTGCTTCCATTG-3’) and B (5’-GAGATTITTTGAGCCCTGCTGTG-3’) were used for amplification of the 1757-bp and 3481-bp for HP1 and HP2 allele-specific sequences, respectively. The primers C (5’-CCTGCCTGATTAACTGCACCAT-3’) and D (5’-CCGAGTGCTCCACATGCATGTTG-3’) were used to amplify a 349-bp HP2 allele-specific sequence.\(^{25}\) Hp genotypes were determined by polymerase chain reaction (PCR) using 20μL total volume reactions containing 2 U/μL of Taq polymerase, 1-100 ng of DNA, and 200μM deoxyribonucleotide triphosphatess (dNTPs) mix; PCR buffer was used as suggested by the supplier (Fermentas Co, Canada). After initial denaturation at 95°C for 5 min, the two-step thermo cycling procedure consisted of denaturation at 94°C for 45 second and annealing at 62°C for 1 min (in the presence of primers A, B, C, and D), and extension at 72°C for 1 min, repeated for 35 cycles, and followed by a final extension at 72°C for 7 min. The thermocycler instrument used was Veriti 96 well (Applied Biosystems, USA). The PCR products for determining HP genotype were separated on 1% agarose gel.

Data was analysed using SPSS 21. Kolmogorov-Smirnov test was performed to verify normal distribution of sampling. Paired sample t-test and Chi square test were used to determine relationship between the variables.

Results
Among the 40 patients analysed, genotypic frequencies for HP1-2 and HP2-2 genotypes were 37.5% (n=15) and 62.5% (n=25), respectively. Of the total, 15 (37.5%) subjects were illiterate, 11 (27.5%) had studied up to primary level, and 7 (17.5%) each had secondary and college education (Table-1). The values of weight and BMI (body mass index) in patients were reduced at the end of the study, but these differences were not significant (p>0.05). Hb levels were significantly increased at the end of the
Fe: Iron.
TIBC: Total Iron Binding Capacity
hs-CRP: high-sensitivity C-reactive protein
SD: Standard deviation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Condition</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
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<td>HP2-2</td>
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<td>62.5</td>
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<td>2</td>
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</tr>
<tr>
<td></td>
<td>Stage3</td>
<td>19</td>
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<td></td>
<td>Stage4</td>
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<tr>
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<td>Secondary</td>
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<td>17.5</td>
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<tr>
<td></td>
<td>Collegiate</td>
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<td>17.5</td>
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<td>History of alcohol use</td>
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<tr>
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<td></td>
<td>Farmer</td>
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<tr>
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<td>22.5</td>
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</table>

No.= Number.

Table-2: The levels of haematological and biochemical factors in patients during study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD (At the beginning of study)</th>
<th>Mean±SD (At the end of study)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (ng/ml)</td>
<td>5.51 ± 5.71</td>
<td>12.33 ± 27.68</td>
<td>0.13</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>9.63 ± 10.48</td>
<td>8.17 ± 19.77</td>
<td>0.65</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.38 ± 1.1</td>
<td>12.05 ± 1.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>268.24 ± 295.64</td>
<td>220.8 ± 254.38</td>
<td>0.17</td>
</tr>
<tr>
<td>TIBC (mg/dl)</td>
<td>356.12 ± 67.99</td>
<td>359.55 ± 82.66</td>
<td>0.07</td>
</tr>
<tr>
<td>Fe (mg/dl)</td>
<td>60 ± 45.12</td>
<td>76.8 ± 22.85</td>
<td>0.57</td>
</tr>
</tbody>
</table>

* Significant vs. the beginning of study (p<0.05)
SD: Standard deviation
hs-CRP: High-sensitivity C-reactive protein
TIBC: Total iron-binding capacity
Fe: Iron.

Table-3: The levels of haematological and biochemical factors in patients based on type of haptoglobin (HP) genotypes.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD (In HP1-2 genotype)</th>
<th>Mean±SD (In HP2-2 genotype)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (ng/ml)</td>
<td>6.75 ± 6.28</td>
<td>4.76 ± 5.34</td>
<td>0.29</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>10.5 ± 11.94</td>
<td>9.1 ± 9.37</td>
<td>0.69</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>11.1 ± 1.1</td>
<td>11.5 ± 1.06</td>
<td>0.26</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>258.5 ± 315.1</td>
<td>274.09 ± 289.87</td>
<td>0.87</td>
</tr>
<tr>
<td>TIBC (mg/dl)</td>
<td>349.93 ± 56.33</td>
<td>359.84 ± 74.98</td>
<td>0.66</td>
</tr>
<tr>
<td>Fe (mg/dl)</td>
<td>60 ± 23</td>
<td>80.72 ± 53.22</td>
<td>0.16</td>
</tr>
</tbody>
</table>

SD: Standard deviation
hs-CRP: high-sensitivity C-reactive protein
TIBC: Total Iron Binding Capacity
Fe: Iron.

Discussion
Anaemia is one of the main problems in CKD patients and occurs especially in the final stages of the disease. Although many attempts have been made to advance the treatment of anaemia in CKD patients, most studies indicate that Hb is low in these patients.7,26,27 For the first time, our results showed that supplementation with LC plus CLA significantly increased Hb levels in CKD patients. Very few studies have been carried out to assess the effects of CLA on anaemia. However, inconsistent with our study, a study has shown that CLA can produce acute oxidative damage and degrade red blood cells.28 Regarding LC supplementation on CKD, several studies have been shown that it may benefit for CKD patients.15,29-31 Hurot et al. showed that LC supplementation can increase Hb and HCT levels and...
decrease erythropoietin requirement in haemodialysis patients. Unlike, Emami et al. showed that LC supplementation can decrease erythropoietin requirement in haemodialysis patients but it does not effect haemoglobin levels in these patients. Another study has shown that LC supplementation can prolong red blood cell (RBC) lifespan and can stimulate erythropoiesis by increasing the number of erythroid colonies. In addition, antioxidant and antiapoptotic effects of LC have also been described both in vitro and in vivo, as well as, it has been shown that LC supplementation can increase heme oxygenase-1 (HO-1) expression and improve erythropoiesis. Thus, regarding the effects of LC and CLA supplementation on haemoglobin levels and erythropoiesis in previous studies, results of our study revealed that LC plus CLA supplementation may be more effective in increasing haemoglobin levels than alone LC or CLA supplementation.

Besides, a common complication in patients with CKD is inflammation. Serum inflammatory markers such as CRP are higher in CKD patients than healthy individuals. Inflammation can increase anaemia, cardiovascular diseases, malnutrition and cancer. It has been shown that inflammatory cytokine response through release of cytokines such as IL-6 and tumour necrosis factor-alpha (TNFα), stimulate the synthesis and release of CRP. In this study, oral LC plus CLA supplementation had no effect on serum levels of inflammatory markers including hs-CRP, IL-6 and ferritin in CKD patients. Similarly, Dehghan et al. showed that the serum levels of CRP didn't change by oral LC supplementation in haemodialysis patients. In contrast to the present study, Duranay et al. reported that carnitine supplementation decreased CRP levels and improved the chronic inflammation in haemodialysis patients. Considering the effect of CLA on inflammation in human population a few studies have been investigated. Similar to our results in the present study, Raff et al. demonstrated that CLA supplementation (5.5 g/d) did not affect inflammatory markers in healthy young men. Unlike our study, Steck et al. showed that CLA supplementation for 12 weeks increased inflammation markers including CRP in obese humans. Also, LaRosa et al. indicated that CLA supplementation associated with increasing in inflammation response in rats. In another clinical trial, Mohammadzadeh et al. confirmed that CLA supplementation reduced inflammatory factors including hs-CRP and IL-6 in rectal cancer patients undergoing chemoradiotherapy. Despite the fact that CLA and LC supplementation may have been benefit or harmful effects on inflammatory response, in our study CLA plus LC supplementation did not effect on inflammatory response in CKD patients and it cannot be use for inflammation controlling in these patients.

Another of our interesting finding was HP2-2 genotype as more frequent genotype among patients with CKD (62.5%). In agreement with our results, Armaly et al. showed that HP2-2 genotype was the prevalent genotype (73%) in CKD patients. Moreover, their results indicated significant association between HP2-2 genotype and CKD. Also, another study has reported the association between HP polymorphism and CKD. It has been shown that the HP2-2 genotype is the prevalent genotype in variety of common disorders including cancer, renal failure and cardiovascular disease. There are several reasons to explain the high frequency of the HP2-2 genotype including high levels of oxidative stress and low serum levels of haptoglobin in this genotype. In addition, in our study, there was no relationship between HP genotype with inflammatory markers. The lack of association between inflammatory markers and HP genotype has not been demonstrated in previous studies. Recently, Mohieldein et al. found no significant association between HP genotype and markers of inflammation in diabetes. Thus, according to the present study, it may indicate that there is no connection between inflammatory markers and HP genotype, which requires further investigation.

The level of literacy and health literacy plays an important role in the care of CKD. In our study, most people with renal disease were illiterate. Our results are consistent with the findings of Taheri et al., which showed that illiteracy is higher in some kidney disease such as haemodialysis patients. Knowledge and education level have a significant impact on the diagnosis and treatment of CKD. Findings of Green et al. showed that level of education is directly associated with better health outcomes in patients with kidney disease. Therefore, educating people to recognise the risk factors and healthy eating plays an important role in preventing kidney disease.

The current study has some limitations. The sample size was relatively small. Other limitations included follow-up of patients to take medications during treatment.

**Conclusion**

This study showed for the first time that supplementation with LC plus CLA was effective in increasing haemoglobin (Hb) levels in CKD patients with anaemia. LC plus CLA supplementation had no effect on BMI and serum levels of IL-6, hs-CRP, TIBC and ferritin.
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Conflict of Interest: None.

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References


