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Influence of L-citrulline supplementation along with conventional treatment and its effect on inflammatory markers in patients with non-alcoholic fatty liver disease

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Abstract

This study evaluated the effect of L-citrulline supplementation combined with conventional therapy on inflammatory and hepatic markers in patients with non-alcoholic fatty liver disease (NAFLD). A total of 110 patients aged 35-45 years were enrolled in a 12-week quasi-experimental study and divided into two groups: G0 (conventional treatment only) and G1 (conventional treatment plus 2 g/day L-citrulline). Anthropometric measurements and biochemical parameters, including lipid profile, liver function tests, C-reactive protein (CRP), and tumor necrosis factor (TNF)-α, as well as dietary intake, were collected at baseline and post-intervention. The mean age was 42.03±4.89 years in G0 and 44.7±4.22 years in G1. Body mass index (BMI) and weight did not change significantly in either group. L-citrulline supplementation in G1 significantly reduced low-density lipoprotein and total cholesterol levels and improved high-density lipoprotein ($p<0.05$). TNF-α levels also decreased significantly in the treatment group ($p=0.0032$), whereas CRP and aspartate aminotransferase showed no significant changes. These findings indicate that L-citrulline supplementation, as an adjunct to conventional therapy, improves inflammatory and lipid parameters in NAFLD patients, particularly TNF-α, low-density lipoprotein, and total cholesterol, without affecting BMI, weight, or CRP. Future multicenter randomized studies are recommended to confirm these results.

Key words: L-citrulline, inflammatory markers, non-alcoholic fatty liver disease, supplementations, liver function test.

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Introduction

The term "non-alcoholic fatty liver disease" (NAFLD) describes a group of disorders characterized by excessive fat accumulation in the liver. The most common form of NAFLD is simple fatty liver,[1] in which fat deposits in liver cells. Although this fat accumulation is abnormal, it usually does not cause liver damage on its own.[2] A small proportion of NAFLD patients may develop non-alcoholic steatohepatitis (NASH),[3] a more severe form in which fat accumulation is accompanied by liver cell inflammation and varying degrees of fibrosis.[4] Globally, approximately 25% of people are affected by NAFLD, which is associated with metabolic conditions such as diabetes, obesity, hyperlipidemia, and hypertension.[5] About 50% of NAFLD cases have NASH, which can cause fibrosis, cirrhosis, or even hepatocellular cancer. NAFLD is a condition that affects non-obese persons in this area as well as obese ones.[6] Hepatocellular carcinoma was caused by NASH in about 11.11% of cases. Patients with NAFLD have a high incidence of diabetes and coronary artery disease. NAFLD is evolving into a future issue for South Asia.[5]

Citrullus vulgaris, the Latin word for watermelon, which has a high concentration of this amino acid, is where the name L-citrulline (Cit) originates.[7] Recent research has, however, highlighted the significance of this amino acid in cellular metabolism as well as in the monitoring of organ functionality.[8] Cit is a precursor to arginine; thus, it is also being looked into as a supplement that can be used to make arginine.[9,10] Cit supplementation is recommended as one of the most effective ways to treat NAFLD's hepatic steatosis and insulin resistance (IR).[11] Through their effects on glucose tolerance, IR, and lipid metabolism, it may reduce hepatic steatosis. Cit may have beneficial effects on decreasing inflammatory indicators like tumor necrosis factor (TNF) and interleukin-6, which can delay the onset of serious diseases like cirrhosis, according to certain research.[12]

The study aimed to evaluate the influence of L-Cit supplementation on inflammatory markers in patients with NAFLD. The study will also help in therapeutic interventions to reduce the complications of the disease, and the supplementation may reduce the severity of the progression of the disease.

Materials and Methods

A case-control study was conducted from January 2024 to June 2024. Patients with NAFLD of both genders between the ages of 35 and 45 years, and C-reactive protein (CRP) levels >1 mg/L were included. Pregnant or lactating women, patients suffering from other health disorders such as hypertension and diabetes, and those with any known allergies such as rash, vomiting, diarrhea, itching, or shortness of breath were excluded.

Screening

During the screening phase, the baseline data were taken from the patients who had been diagnosed with NAFLD by the physician and met the inclusion criteria. It was comprised of anthropometric measurements [weight, height, body mass index (BMI)], blood lipid profile, and inflammatory markers, liver function tests, including average alanine transaminase (ALT) and aspartate aminotransferase (AST), CRP, and TNF-α. Weight was taken by a weighing scale, and height was measured by a stadiometer/measuring tape, in order to calculate BMI. The biochemical data were taken from the lab reports (blood test) of the patients. Blood samples were collected by the hospital lab assistant to assess the effect of L-Cit supplementation among patients.

Consent

After explaining the benefits of the study to all participants and explaining that they will be free to withdraw from the study at any stage in the study, taking prior written informed consent in written form, the data were collected by the researcher with the help of a self-administered questionnaire/proforma. All the participants were informed and ensured that their personal data/identity would not be revealed, and the collected data would be processed for publication. Ethical approval was taken from the Ethical Committee of the University of Lahore.

Allocation

Participants suffering from NAFLD were selected from the District Headquarter Hospital, Dera Gazi Khan, after obtaining permission from the relevant authorities. The study followed a quasi-experimental (non-randomized case-control) design. Eligible participants were divided into two groups based on matching criteria, including age (±3 years), gender, and baseline CRP levels, to minimize bias. A total of 110 participants were randomly selected into two groups according to the sequence of recruitment: the conventional treatment group (G0, n=55), *i.e.*, particular standard treatment according to the recommendation of the physician, and the treatment group (G1, n=55), *i.e.*, a 2 g of L-Cit supplements daily in addition to regular treatment as recommended by the physician. This assignment was to provide a fairly balanced group of participants in both groups.[13] After adding the dropout rate, 55 participants were allocated to each group. The follow-ups for patients were conducted once a week. The anthropometric measurements were collected in each follow-up. Patients were asked about any constraints and barriers to following the study procedure.

Statistical analysis

IBM SPSS version 27.0 (IBM, Armonk, NY, USA) was used for descriptive and t-test analysis between the study groups.

Results

The mean age statistics of NAFLD patients enrolled in the study are presented in Table 1. The mean age of G0 (conventional group) participants was 42.03 with a standard deviation of ±4.89, and the mean age of G1 (treatment group) participants was 44.7 with a standard deviation of ±4.22.

The mean CRP level of participants before treatment in G0 was 13.6 with a standard deviation of ±2.37 mg/L, and in G1, 14.8 with a standard deviation of ±4.30 mg/L and mean CRP level of participants after treatment in G0 was 16.89 with a standard deviation of ±3.03 mg/L, and in G1, 11.56 with a standard deviation of ±3.26mg/L. The median is 56 (27) and 59 (29) for G0 and G1 before treatment, whereas the median after treatment is 48 (25) for G0 and 55 (26) for G1. Both groups showed no statistically significant difference in pre- and post-treatments with p-values of 0.12 and 0.62, respectively (Table 2).

Table 1. Average age distribution of non-alcoholic fatty liver disease patients enrolled in the study.

Treatments	Age (Mean±SD)	Minimum	Maximum
G0	42.032±4.89	35	43
G1	44.7±4.221	35	45

SD, standard deviation; G0, conventional group; G1, treatment group.

Table 2. Comparison of the average C-reactive protein (CRP) level pre- and post-treatment.

Treatments		Mean±SD	Median (IQR)	Mean±SD difference (F)	Wilcoxon (p)
Conventional group (G0)	Pre	13.6±2.37	56 (27)	-3.29±0.66	0.12
	Post	16.89±3.03	48 (25)		
Treatment group (G1)	Pre	14.8±4.30	59 (29)	3.24±1.04	0.062
	Post	11.56±3.26	55 (26)	3.24±1.04	0.032

SD, standard deviation; IQR, interquartile range.

The mean TNF-α level of participants before treatment in G0 was 16.75 with a standard deviation of ±1.33 pg/dL and in G1, 18.6 with a standard deviation of ±2.76 pg/dL and mean TNF-α level of participants after treatment in G0 was 17.98 with a standard deviation of ±1.61 pg/dL and in G1, 15.3 with a standard deviation of ±2.14 pg/dL. The median is 16 (7) and 17 (4) for G0 and G1 before treatment, whereas the median after treatment is 16 (7.5) for G0 and 16 (4) for G1. The treatment group showed a statistically significant difference in pre- and post-treatments with a p-value of 0.0032 (Table 3).

The mean ALT level of participants before treatment in G0 was 68.8 with a standard deviation of ±6.34 U/L, and in G1, 52.65 with a standard deviation of ±4.12 U/L and mean ALT level of participants after treatment in G0 was 66.56 with a standard deviation of ±5.94 U/L, and in G1, 52.97 with a standard deviation of ±6.7 U/L. The median is 49 (39) and 58 (39) for G0 and G1 before treatment, whereas the median after treatment is 49 (25) for G0 and 41 (35) for G1. The treatment group showed a statistically significant difference in pre- and post-treatments (0.01), respectively (Table 4).

The mean AST level of participants before treatment in G0 was 93.0 with a standard deviation of ±7.8 U/L, and in G1, 74.7 with a standard deviation of ±4.96 U/L, and the mean in G1 was 67.4 with a standard deviation of ±3.4 U/L. The median is 56 (27) and 59 (29) for G0 and G1 before treatment, whereas the median after treatment is 48 (25) for G0 and 55 (26) for G1. Both groups showed no statistically significant difference in pre- and post-treatments with a p-value of 0.12 (Table 5).

Discussion

The mean AST level of participants before treatment in G0 was 93.0 with a standard deviation of ±7.8 U/L, and in G1, 74.7 with a standard deviation of ±4.96 U/L, and the mean AST level of participants after treatment in G0 was 95.63 with a standard deviation of ±7.67 U/L, and in G1, 67.4 with a standard deviation of ±3.4 U/L. The median is 56 (27) and 59 (29) for G0 and G1 before treatment, whereas the median after treatment is 48 (25) for G0 and 55 (26) for G1. Both groups showed no significant differences in pre-treatment values (p=0.12 and 0.62). Participants received four 500 mg capsules daily (L-Cit or placebo) and were advised to follow a balanced diet and engage in physical activity. Liver enzymes, hepatic steatosis, and fibrosis were assessed at baseline and after the intervention. Post-treatment, the Cit group showed a significant reduction in ALT and hepatic steatosis compared to the control group ($p<0.05$). Furthermore, serum levels of AST showed no significant differences between the two groups ($p\geq0.05$).[13] The mean CRP levels of NAFLD patients before and after treatment are presented in Table 2. The mean CRP level of participants before treatment in G0 was 13.6±2.37 mg/L and in G1, 14.8±4.30 mg/L, and the mean CRP level after treatment in G0 was 16.89±3.03 mg/L and in G1, 11.56±3.26 mg/L. Both groups showed no statistically significant difference in pre- and post-treatment values, with p-values of 0.12 and 0.62, respectively. This finding is critically important because TNF-α is not merely a marker but a key driver of inflammation in NAFLD, promoting liver cell injury and fueling the progression from simple steatosis to the more severe necroin-

Table 3. Comparison of the average tumor necrosis factor-α (TNF-α) level pre- and post-treatment.

Treatments		Mean±SD	Median (IQR)	Mean±SD difference (F)	Wilcoxon (p)
Conventional group (G0)	Pre	16.75±1.33	16 (7)	-1.23±0.28	0.07
	Post	17.98±1.61	16 (7.5)		
Treatment group (G1)	Pre	18.6±2.76	17 (4)	3.3±0.62	0.0032
	Post	15.3±2.14	16 (4)		

SD, standard deviation; IQR, interquartile range.

Table 4. Comparison of the average alanine transaminase (ALT) level pre- and post-treatment.

Treatments		Mean±SD	Median (IQR)	Mean±SD difference (F)	Wilcoxon (p)
Conventional group (G0)	Pre	68.8±6.34	49 (39)	2.24±0.4	0.53
	Post	66.56±5.94	49 (25)		
Treatment group (G1)	Pre	52.65±4.12	58 (39)	0.32±2.58	0.01
	Post	52.97±6.7	41 (35)		

SD, standard deviation; IQR, interquartile range.

Table 5. Comparison of the average aspartate aminotransferase (AST) level pre- and post-treatment.

Treatments		Mean±SD	Median (IQR)	Mean±SD difference (F)	Wilcoxon (p)
Conventional group (G0)	Pre	93.0±7.8	56 (27)	-2.63±0.13	0.12
	Post	95.63±7.67	48 (25)		
Treatment group (G1)	Pre	74.7±4.96	59 (29)	7.3±1.56	0.062
	Post	67.4±3.4	55 (26)		

SD, standard deviation; IQR, interquartile range.

flammation characteristic of NASH. The observed reduction suggests that L-Cit supplementation may directly target a central inflammatory pathway in the disease. This effect is potentially mediated through L-Cit's role as a precursor to L-arginine and nitric oxide (NO), as enhanced NO signaling is known to suppress the activation of the pro-inflammatory nuclear factor κB (NF-κB) pathway, a primary regulator of TNF-α production. Therefore, the significant decrease in TNF-α indicates a potential mechanism by which L-Cit could alter the disease course and halt its progression. The treatment group showed a statistically significant difference in pre- and post-treatment TNF-α levels with a p-value of 0.0032. In a study, Darabi *et al.* explored the impact of L-Cit supplementation on inflammatory markers in people with NAFLD. In their clinical research, they split 50 patients into two groups; the first group received a placebo, and the second group received daily supplements containing 2 g of Cit. The results demonstrated that the intervention group's serum levels of NF-κB and high-sensitivity CRP were significantly lower than those of the placebo group. Their findings indicate that supplementing with 2 g/day of Cit for 12 weeks reduces inflammatory markers in NAFLD patients.[14] The single-center, non-randomized design of the present study may limit the generalizability of findings and introduce selection bias. Moreover, the discussion essentially reiterates the results without providing interpretative insights regarding TNF-α, the only parameter that significantly changed with L-Cit supplementation. Future multicenter randomized studies are recommended to confirm these findings and further explore the mechanistic effects of L-Cit in NAFLD management.

Conclusions

L-Cit supplementation in combination with traditional treatment induced better hepatic and inflammatory biomarkers in NAFLD patients, as they demonstrated significant decreases in TNF-α and ALT levels. CRP, BMI, and weight did not show any significant change. Its impact on lipid metabolism cannot be determined as the lipid profile data were not available. These results should be confirmed by further multicenter randomized studies.

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Informed consent: prior written informed consent was obtained from all the study participants.

Patient consent for publication: all the participants were informed about the benefits and risks of the study, and prior consent was taken about publishing the data while keeping their personal information anonymous.

Availability of data and materials: data are available upon reasonable request made to the corresponding author.