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The effectiveness of L-Carnitine on improving liver enzymes in Leukemic patients treated with Methotrexate and 6-Mercaptopurine

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Abstract

Background: One of the side effects of Methotrexate and 6-Mercaptopurine used in the treatment of patients with Acute Lymphoblastic Leukemia (ALL) is drug hepatitis increasing the level of liver enzymes. The aim of this study was to determine the effectiveness of L-Carnitine in improving liver enzymes in patients treated with methotrexate and 6-Mercaptopurine.

Methods: This study was conducted as a single-blind randomized clinical trial (RCT) in the pediatric oncology department of Mohammad Kermanshahi Hospital in Kermanshah. It was performed on 26 patients with ALL treated with methotrexate and 6-mercaptopurine who their liver enzymes were between two to four times normal level. Patients were randomly assigned into two groups including the L-carnitine receiving group and the placebo receiving group. Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels and L-carnitine side effects including headache, vomiting, and diarrhea were assessed Up to 3 month, once every two weeks. To analyze the data, SPSS was used and the significance level was considered at < 0.05.

Results: There were no statistically significant differences between control and intervention groups in terms of side effects of L-carnitine including diarrhea, vomiting and headache (P-value> 0.05). In the first four visits, no statistically significant difference was observed in the level of liver enzymes in the two groups (P-value> 0.05). However, in the fifth and sixth evaluations, a statistically significant difference was seen at the level of liver enzymes between the two groups of intervention and control (P-value <0.05).

Conclusion: Based on the results of this study, it seems that L-carnitine in patients treated with methotrexate and 6-mercaptopurine is effective in improving the level of liver enzymes

Key Words: ALL, L-Carnitine, Liver Enzymes, Methotrexate ,6-Mercaptopurine.

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1- INTRODUCTION

Leukemia is the most common malignant neoplasm of childhood, accounting for about 31% of all malignancies in children under the 15 years of age. From all types of pediatric leukemia, Acute Lymphoblastic Leukemia (ALL) accounts for approximately 77% of diagnoses. Acute Myelogenous Leukemia (AML) comprises about 11% of childhood leukemia, Chronic Myelogenous Leukemia (CML) about 2 to 3%, and Juvenile Myelomonocytic Leukemia (JMML) accounts for almost 1 to 2% (1).

Childhood ALL was the first progressive cancer that has been shown to be treatable. It was also the first type of malignancy that it's principles of diagnosis, prognosis, and treatment were found. In fact, the disease involves a heterogeneous group of malignancies with various genetic disorders, leading to a variety of clinical manifestations and responses to different therapies. The maximum prevalence of the disease is between 2 and 3 years, and is slightly higher in boys than in girls (1). Maximum significant time of disease prevalence is between the ages of 2 and 3 years, and is slightly higher in boys than in girls (1).

About 2,400 ALL cases are being diagnosed in children under the age of 15 in the United States each year. The disease is more common in children with certain chromosomal abnormalities, such as Down syndrome, Bloom's syndrome, Ataxia-telangiectasia, and Fanconi syndrome. Among identical twins, the risk of disease in the second twin is higher than in the general population if one of the twins has leukemia (1).

Methotrexate is an anti-metabolic drug that is structurally an analogous of folic acid (2). The dose of methotrexate in patients with acute lymphoblastic leukemia is 20 mg per square meter of body surface (3). The 6-Mercaptopurine is also an analogue of hypoxanthine and along with methotrexate is the selective drug in the treatment of acute leukemia in children, especially, in cases where a long course of treatment is required (4). The dose of 6-mercaptopurine in acute lymphoblastic leukemia is 50 mg per square meter of body surface area (3).

L-Carnitine (3-methylamino-butyric acid) is a derivative of the amino acids lysine, methionine, and nutrients found in meat and dairy products; a pseudo vitamin needed for the body (5). L-carnitine is produced 75% in food sources and 25% endogenously from essential lysine and methionine amino acids in the human body, releasing energy from fat cells (6). L-carnitine has very important physiological roles, including the transfer of long-chain fatty acids (LCFA) from the inner mitochondrial membrane (IMM) to beta-oxidation and the production of adenosine triphosphate (ATP) in peripheral tissues. L-Carnitine prevents oxidative stress and regulates nitric acid, cellular respiration and the activity of enzymes involved in oxidative stress (6).

In children with acute lymphocytic leukemia, cytotoxic drugs such as methotrexate and 6-mercaptopurine are used to prevent recurrence. However, one of the most important side effects of these drugs is their effect on liver enzymes. L-carnitine appears to reduce the side effects of chemotherapy drugs. This study was, then, conducted to investigate the effectiveness of L-carnitine in improving liver enzymes in patients treated with methotrexate and 6-mercaptopurine. If L-carnitine is proven to be effective in these patients, an effective action can be taken to reduce the side effects of chemotherapy drugs on the liver.

2- METHODS

The present study was a single-blind RCT that was performed for three months from October to December 2018 in the

oncology clinic of Dr. Mohammad Kermanshahi Hospital in Kermanshah city, located in the western part of Iran. Patients were children with acute lymphocytic leukemia who were receiving maintenance treatment with methotrexate and 6-mercaptopurine, and their liver enzymes were between two to four times normal levels. Because patients were under 18 years of age, for their participation in the study, a written consent form was obtained from their parents. The exclusion criteria were liver enzymes over four times normal levels, clinical hepatitis, hypothyroidism, convulsions, and L-carnitine intolerance. The sample size was calculated as 13 patients in each group considering $\alpha = 0.05$, power of 90%, and using the appropriate formula. The samples were divided into two groups by the systematic random sampling method. This study received the approval of ethics from the ethics committee of Kermanshah University of Medical Sciences and prior to being performed, its steps and objectives were explained to the parents. The 26 ALL patients treated with methotrexate and 6-mercaptopurine were randomly divided into two groups including L-carnitine-receiving and placebo receiving groups. The groups were matched in terms of age and gender. In the intervention group, patients received 50 mg daily L-carnitine tablets or syrup per kg body weight under the supervision of a specialist, and in the control group, placebo made by the same company, which was completely similar in shape, color and size to the main drug was given for 3 months. Patients in both groups also received folic acid supplements. Then, once every two weeks, patients' liver enzymes including ALT and AST levels were evaluated, and patients were monitored for side effects such as headache, diarrhea, and vomiting and if necessary the doses were changed. To measure biochemical variables, a 5 ml blood sample was taken from the patients after 12 hours of fasting. The patients' information was collected through interviews and the test results were entered in the relevant checklist. Independent t-test and chi-square test were used to compare baseline and demographic characteristics between the two groups. Friedman test was used to examine the changes in liver enzymes in both groups; and Mann-Whitney and independent t-test were used to compare the two groups in each time interval. . Data analysis was performed using SPSS software version 16 and the p-value less than 0.05 was considered to be significant.

3- RESULTS

A sample of 26 children with ALL, being treated with methotrexate and 6-mercaptopurine, were entered in the study. During the 3-month intervention, 13 children received L-carnitine and others received placebo. The age of patients was between 4 and 14 years with a mean and standard deviation of 7.62 ± 2.81 years. The mean age of patients in L-carnitine and placebo groups was 7.08 ± 2.92 and 8.15 ± 2.7 years, respectively. In terms of gender, L-carnitine group included 8 boys and 5 girls and placebo group included 7 boys and 6 girls.

Demographic characteristics of patients are shown in **Table 1**. Based on the results, the patients in the two groups were similar in terms of age and gender distribution, and according to the appropriate statistical test there was no statistically significant difference between the age and gender of the patients in the intervention and control groups (P-value <0.05).

Table-1: Distribution/frequency of age and gender variables in L-carnitine and placebo groups

Variables	Groups		p-value
	L-carnitine	Placebo	
Age	7.08 ± 2.92	8.15 ± 2.7	0.0340*
Boys	8	7	0.0433**
Girls	5	6	

*Independent T-test
**Chi-square test

Mean and standard deviation of quantitative values of ALT and AST parameters in the whole study sample before and after treatment based on Kolmogorov-Smirnov test did not have a normal distribution. Therefore, to compare the quantitative values of ALT and AST parameters in the two groups in each time evaluation, we used Mann-Whitney test, and Friedman nonparametric test was used to compare the trend of changes in each group after treatment. As shown in **Tables 2 and 3**, the mean of the quantitative variables between the two groups of L-carnitine and placebo receiving were compared at different times after treatment.

Table-2: Changes in AST levels in the two groups of L-carnitine and placebo; from the first to the sixth evaluations

Variable	Evaluations	Groups		p-value
		L-carnitine	Placebo	
		Mean ± SD	Mean ± SD	
AST	2 weeks later	48.46 ± 21.801	46.15 ± 33.385	0.293
	4 weeks later	47.85 ± 19.540	61.38 ± 31.264	0.200
	6 weeks later	111.23 ± 34.308	59.77 ± 46.009	0.918
	8 weeks later	44.92 ± 23.571	77.38 ± 64.214	0.199
	10 weeks later	59.08 ± 54.894	107.38 ± 92.775	0.043
	12 weeks later	39.77 ± 21.676	85.85 ± 68.442	0.007
p-value		0.017	0.420	-

The results showed that there was no statistically significant difference in the levels of liver enzymes AST and ALT between the experimental and control groups, in the first four visits (P-value <0.05). However, in the next visits, i.e. 10 weeks and 12 weeks after the intervention, a statistically significant difference was observed in the levels of liver enzymes between the experimental and control groups (P-value <0.05).

Table-3: Changes in ALT levels in the two groups of L-carnitine and placebo; from the first to the sixth evaluations

Variable	Evaluations	Groups		p-value
		L-carnitine	Placebo	
		Mean ± SD	Mean ± SD	
ALT	2 weeks later	82.38 ± 62.037	73.85 ± 93.109	0.330
	4 weeks later	79.15 ± 49.99	79.15 ± 57.984	0.778
	6 weeks later	83.62 ± 75.97	111.23 ± 91.634	0.521
	8 weeks later	61.69 ± 43.50	105.23 ± 68.767	0.066
	10 weeks later	59.08 ± 55.68	104.23 ± 46.691	0.035
	12 weeks later	51.38 ± 25.260	99.54 ± 66.439	0.027
p-value		0.015	0.496	-

The analysis of the findings also showed that the trend of changes in the mean levels of liver AST and ALT enzymes in the L-carnitine group was significant, while no significant change was observed in the placebo group (**Figure 1 and 2**).

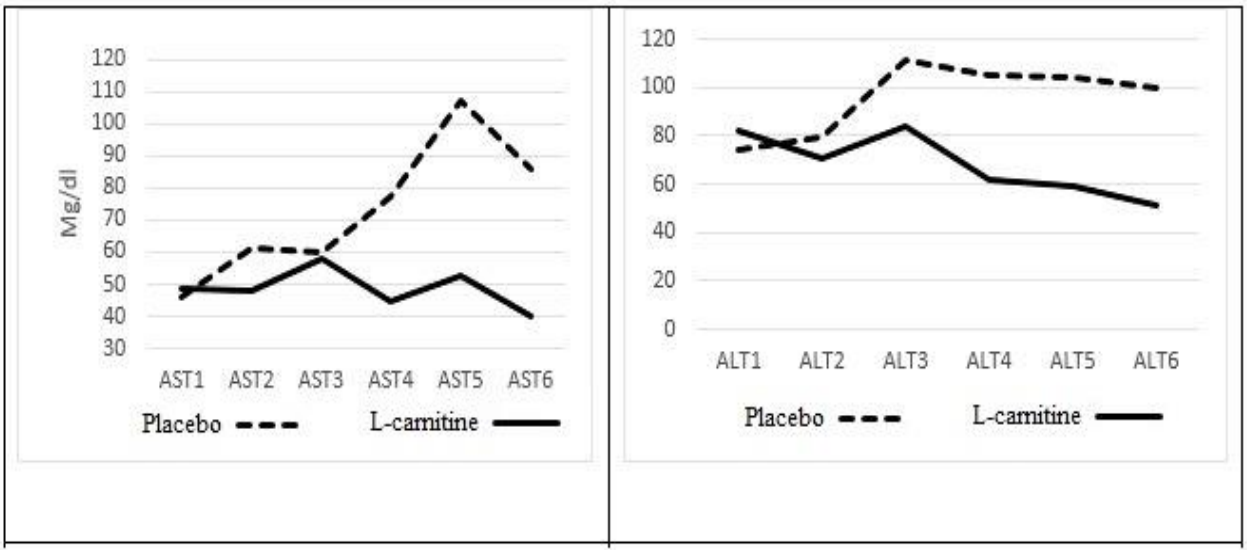

Fig. 1: Linear graph comparing the mean AST levels between the two groups of L-carnitine and placebo

Fig. 2: Linear graph comparing the mean ALT levels between the two groups of L-carnitine and placebo

The results of Cochran test show that the incidence of headache, diarrhea and vomiting in the control and intervention groups are not significantly different.

4- DISCUSSION

Methotrexate and 6-mercaptopurine are used to treat acute lymphoblastic leukemia, and one of the side effects of these drugs is an increase in liver enzymes.

The aim of this study was to determine the effect of oral L-carnitine in patients treated with methotrexate and 6-mercaptopurine. AST and ALT levels were compared in the intervention and control groups. In the first four visits, there was no statistically significant difference in the levels of liver enzymes in the control and intervention groups. However, in the fifth and sixth evaluations, a statistically significant difference was observed in the levels of liver enzymes between the two groups. These findings are consistent with the results of the study of Hashemi et al, in 2010 (7) and also the study of Tousson et al, in 2014 (8). Studies such as those by Hong et al, (9) and Abdel-Ghaffar et al, (10) that were performed on male rats have also confirmed this result. In our study, oral L-carnitine consumption in patients treated with methotrexate and 6-mercaptopurine was effective in improving liver enzymes, although this effect was related to the duration of L-carnitine use.

In terms of demographic factors such as age and gender, there was no statistically significant difference between the intervention and control groups. Complications of L-carnitine including headache, vomiting and diarrhea were evaluated and compared in the two groups of intervention and control. There was no statistically significant difference in the incidence of side effects in the two groups, and the use of L-carnitine was tolerable for patients.

5- CONCLUSION

Based on the results of this study, it seems that the use of oral L-carnitine in patients treated with methotrexate and 6-mercaptopurine is effective in improving liver enzymes and the side effects of L-carnitine are tolerable.

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