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The Effect of L-Glutamine on Pain Crisis Reduction in Patients with Sickle Cell Anemia and Sickle β°-Thalassemia

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Abstract
Background: A low level of L-glutamine, a precursor of nicotinamide adenine dinucleotide (NAD) in red blood cells (RBCs), is identified as an underlying mechanism for the potential decrement of the NAD redox and the incidence of pain crisis in sickle cell anemia (SCA). The aim of this study is to assess the impact of oral L-glutamine therapy on pain crisis reduction in patients with SCA and sickle β°-thalassemia.
Materials and Methods: In this pilot clinical trial, 15 patients with SCA and sickle β°-thalassemia with the mean age of 17.2 ± 6.07 were examined to evaluate the efficacy of L-glutamine therapy in pain crises. All the subjects received oral L-glutamine (0.3 gr /kg of body weight) every day for 8 weeks. With respect to the effects of L-glutamine on pain crisis, the subjects were in contact with the physicians at least once or more per week. Blood samples were taken at different follow-up times to evaluate the laboratory characteristics of the patients.
Results: Pain crises occurred for 1-3 times (mean: 1.29 ± 1.05) during 12 months before the L-glutamine therapy. With no significant change, the patients had lower numbers of painful crises (mean: 0.80 ± 0.58) at the end of a 4-week period compared to the period before the L-glutamine therapy (P = 0.466). Fewer pain crises with a mean of 0.33 ± 0.51 occurred during 8 weeks of L-glutamine therapy than in the pre-treatment period (P = 0.038).
Conclusion: The results of the present study showed that oral L-glutamine can mitigate a pain crisis and improve the sickle RBCs and reticulocyte count in SCA. This suggests that L-glutamine therapy can increase the antioxidant potential of sickle RBCs.
Keywords: L-glutamine, Sickle β°-thalassemia, Sickle cell anemia

Introduction

Sickle cell anemia (SCA) is a common genetic disorder of the β-globin chain which is caused by the displacement of glutamic acid by valine at the sixth position of the amino acid sequence of the β-globin chain (1). SCA is an autosomal recessive disorder inherited in both heterozygote and homozygous forms. The heterozygote form is the most common hemoglobinopathy in the world. It is a completely benign disorder without any clinical signs and hematological abnormalities, while the homozygous form is a chronic and severe hemolytic anemia (2). In the case of homozygotes, hemoglobin (Hb) A is not produced, and the major Hb of the patient is HbS, HbF and a small amount of Hb A2 (2). HbS is freely soluble when it is completely oxygenated. However, when it separates from oxygen, it becomes polymerized, creates fluid crystals and changes red blood cells (RBCs) into sickle cells (3). Patients with SCA usually experience a pain in the chest, abdomen and joints. There are also pulmonary complications as well as hands and feet swelling due to sickle-shaped RBCs that block the blood flow through tiny blood vessels (4, 5). Previous studies have shown that changes in the homeostasis of nicotinamide adenine dinucleotide (NAD), as a redox potential molecule of oxidant stress, play a key role to increase sickle RBCs in patients and

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healthy individuals. In these studies, a low level of L-glutamine, a precursor for NAD production, is introduced as an underlying mechanism for defects in NAD metabolism and the increased level of sickle-shaped RBCs (6). In fact, the NAD redox potential decreased due to the low level of L-glutamine is the main factor for the presence of sickle RBCs in oxidant stress conditions (6-8). Traditionally, hydroxyurea is the first therapeutic approach for adults and children with SCA and β-thalassemia, which is approved by food and drug administration (FDA) (9). Despite its efficacy to reduce morbidity and mortality, hydroxyurea therapy remains the subject of debate due to doubts regarding the impact of various factors, such as the age of SCA patients (10-12). It has been shown that L-glutamine and L-arginine can be considered as the primary treatment for SCA patients who have incurred hydroxyurea (13-15). In addition, through several clinical trials, Niihara et al. (16-18) showed that L-glutamine supplementation can lead to the increased production of NAD, defense against oxidant stresses in sickle RBCs as well as the reduction of pain and hospitalizations. Some of the possible mechanisms leading to these changes relate to the increased activity of the NAD syntheses in the sickle RBCs (19, 20). Since SCA is a common Hb disorder in the southwest of Iran (21) and, in recent decades, L-glutamine has been used as a supplementation to improve the clinical implications of SCA, this pilot clinical trial was conducted to better understand the laboratory characteristics and the potentially beneficial effects of L-glutamine on pain reduction. Increasing the knowledge about the clinical usefulness of L-glutamine can lead to the better management of SCA patients.

Materials and Methods

Study design

This pilot clinical trial was carried out on the patients who referred to Ahvaz Baghay Hospital for SCA and sickle β°-thalassemia. The inclusion criteria for the eligibility of the subjects were the diagnosis of SCA and sickle β°-thalassemia and presence of at least one episode of pain 12 months before the conduction of the study. Also, the exclusion criteria were chronic diseases that could interfere with the participation, pregnancy, transfusion of blood products in the previous three months, hepatic and renal failure, Hb value of less than 5 g/L, history of hospitalization of more than 10 times, history of opioid dependence and glutamine sensitivity. In addition, the patients could leave the study if they needed blood transfusions or other treatments.

Ethical considerations

This study was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1397.121). The IRCT code is IRCT20180603039959N1. Letters of informed consent were also taken from the patients or their parents (for children).

Intervention

After the informed consent was taken, the patients who had all the eligibility criteria received oral L-glutamine at 0.3 grams per kilogram every day for 8 weeks, based on the phase-3 trial of L-glutamine in SCA by Niihara et al (18). The pre-treatment of the pain was determined on the basis of the medical records of the patients during hospitalization. Also, in order to record the number of pain experiences during the study, the investigator physician kept in contact with each subject at least once or more times per week; the participants were visited on a weekly basis for a brief clinical assessment.

With respect to the effect of L-glutamine on pain and laboratory characteristics, a blood sample was collected at the baseline after 4 and 8 weeks of L-glutamine therapy. The sample was kept in ethylene diamine tetra acetic acid (EDTA) containing tubes.

Statistical analysis

The normality of the variables was assessed using the Kolmogorov-Smirnov

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test. Descriptive statistical methods were also used to calculate the mean and standard deviation (SD) of the demographic and other quantitative variables. Paired student's t-tests and repeated ANOVA tests served to evaluate the differences among the variables at different follow-up times with respect to the data (hematological parameters) that had a normal distribution. Besides, since pain crises are not normally distributed, Friedman test was performed to evaluate the differences in this variable at different follow-up times. All the statistical analyses were carried out using SPSS version 22. The P-value of < 0.05 was considered statistically significant.

Results

In this study, a simple random sampling method was used for patient selection. Thus, the individuals with SCA and sickle β°-thalassemia who had referred to Shafa Hospital were adopted as the research population. Based on the inclusion and exclusion criteria, among 26 patients with SCA and sickle β°-thalassemia, 11 patients were not included in this study (Figure 1). The treatment groups having enrolled in this pilot clinical trial included 15 patients (6 females and 9 males with a mean age of 17.2 ± 6.07 years). The baseline demographic data and the number of crises of all the patients during different follow-up times are presented in Table I. To investigate the effect of L-glutamine on pain reduction in patients with SCA and sickle β°-thalassemia, the pain criseis of three periods (i.e., pre-treatment as well as 4 and 8 weeks after L-glutamine therapy) were compared.

The pain episode was 1 to 3 times (mean: 1.29 ± 1.05) during 12 months before the L-glutamine therapy. In addition, a comparison was performed of the numbers of pain crises at different follow-up times. During 4 weeks, the patients showed fewer such crises (mean: 0.80 ± 0.58) than in the period before the L-glutamine therapy. However, as shown in Table II, no significant difference was found in this regard (P = 0.466). Similarly, there was no significant difference between the mean numbers of the pain crises during 4 weeks and 8 weeks of L-glutamine therapy (Table II). As shown in Figure 2, the fewer pain crises during 8 weeks (mean: 0.33 ± 0.51) were significantly different from the case within the time interval before the L-glutamine therapy (P = 0.038). In conjunction with the pain assay, the levels of Hb, RBC and reticulocyte at different follow-up times were analyzed. In this regard, the Hb levels were not significantly different after 4 and 8 weeks of L-glutamine therapy in comparison to the baseline determined by the paired student's t-test (Table II). Also, RBC, hematocrit and reticulocyte were somewhat higher but without significant differences in 4 weeks of L-glutamine therapy in comparison to the baseline. After 8 weeks, RBC, reticulocyte and hematocrit were significantly higher than the baseline (P < 0/05 as set by the paired student's t-test) (Table II). Because of the relatively small size of the studied population, repeated ANOVA tests were used to confirm the changes in the hematological variables during different follow-up times. As shown in Table II, the results obtained were relatively similar to the findings of the paired student's t-test. Compared to the baseline value, the mean of the RBC count significantly increased in 8 weeks of oral L-glutamine therapy (P = 0.043). Also, similar to the results of the paired student's t-test, the mean of reticulocyte and hematocrit was significantly different from the baseline. The P values were 0.039 and 0.019, respectively (Table II).

As for side effects, none such as musculoskeletal pain, fatigue and chest pain was observed at the end of 8 weeks of L-glutamine therapy.

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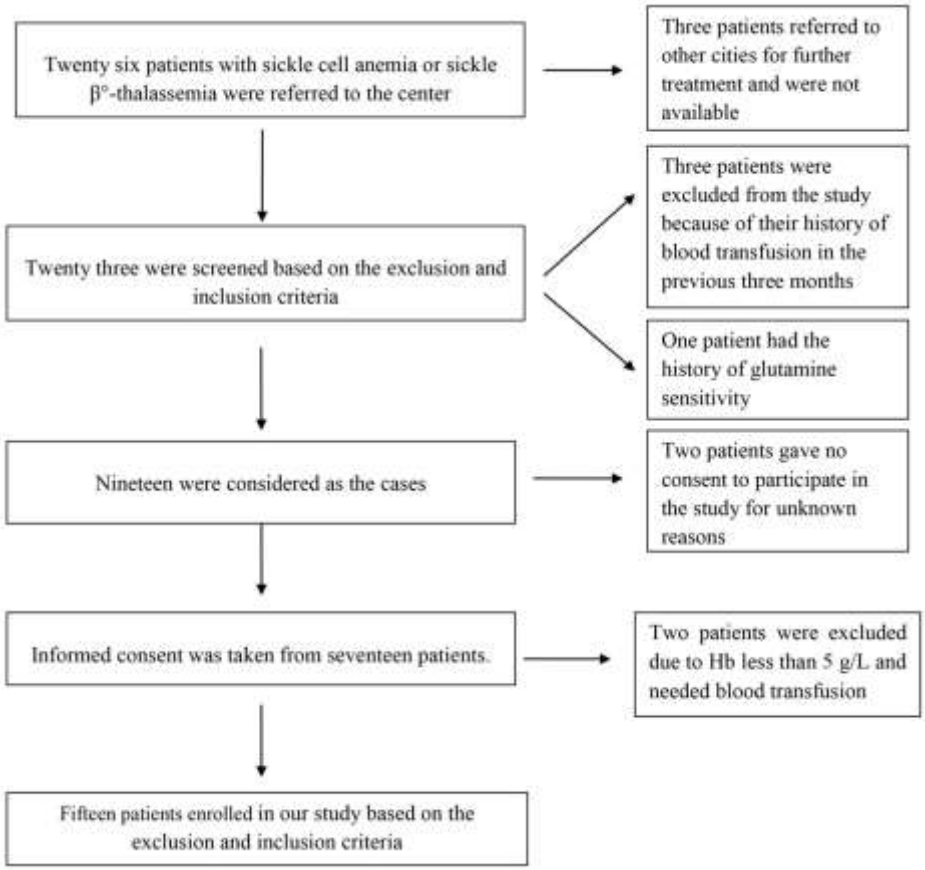

Figure 1. Flow chart of the patient selection based on the inclusion and exclusion criteria

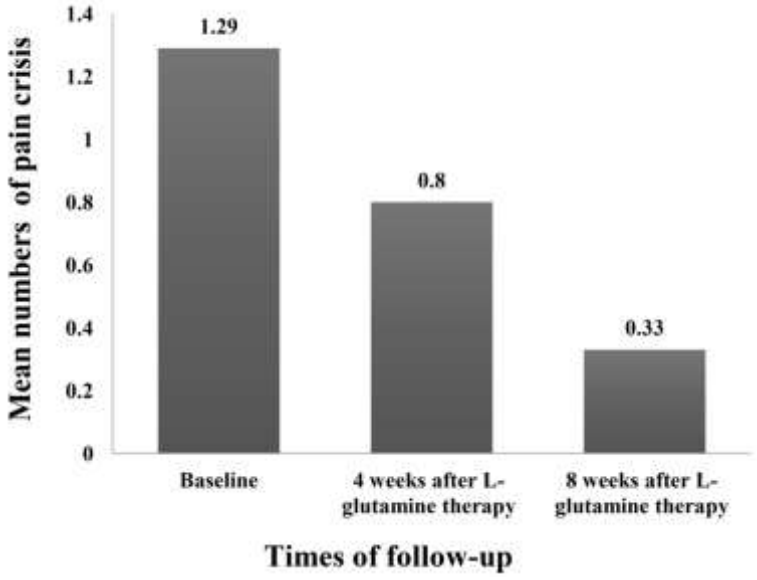

Figure 2. Comparison of the painful episodes during the study: The lower numbers of pain crisis in 4 weeks of L-glutamine therapy did not show any significant differences compared to the time interval before the treatment initiation (P = 0.466) and 8 weeks later (P = 0.564). The mean numbers of pain crisis after 8 weeks were significantly lower than those in the period before the L-glutamine therapy (P = 0.038).

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Table I. Baseline characteristics and number of pain crises within different follow-up times

Characteristics	
Sex **Male** **Female**	**No (%)** 9 (60) 6 (40)
Age (Year) **Min: 5** **Max: 27**	**(Mean ± SD)** 17.2 ± 6.07
Laboratory characteristics **RBC (×10^9/L)** **Hb (g/dL)** **Reticulocyte count (%)** **HCT (%)**	**(Mean ± SD)** 2.73 ± 2.11 8.13 ± 0.98 0.52 ± 0.81 25.01 ± 5.31
Number of pain crises during 12 months before the course **1-2** **2-3** **≥ 3**	***No (%)*** 4 (26.7) 5 (33.3) 6 (40)

Abbreviations: No: Number; **RBC:** Red blood cell; **Hb:** Hemoglobin; **HCT:** Hematocrit; **SD:** Standard deviation.

Table II. Comparison of laboratory characteristics and pain crisis numbers in different follow-up times

Parameters	***Baseline (mean ± SD)***	***4 weeks after L-glutamine therapy (mean ± SD)***	***8 weeks after L-glutamine therapy (mean ± SD)***	***P-Value*** ^{1a} **(95% CI of diff) (Paired student's t-test)**	***P-Value*** ^{2a} **(95% CI of diff) (Paired student's t-test)**	***P-Value*** ^{3a} **(95% CI of diff) (Paired student's t-test)**
				P-Value ^{1b} (95% CI of diff) (Repeated measurement ANOVA test)	***P-Value*** ^{2b} (95% CI of diff) (Repeated measurement ANOVA test)	***P-Value*** ^{3b} (95% CI of diff) (Repeated measurement ANOVA test)
No. of pain crises	1.29 ± 1.05	0.80 ± 0.58	0.33 ± 0.51	0.466 (-0.24 – 1.18)	0. 564 (-0.035 – 0.068)	0.038 (0.33 – 1.53)
RBC (×10^9/L)	2.73 ± 2.11	3.21 ± 1.2	5.4 ± 2.1	0.714 (0.15 – 2.51)	0.061 (-0.32 – 2.41)	0.047 (0.86 – 2.71)
				0.526 (-0.79 – 0.25)	0.071 (-2.62 – 1.65)	0.043 (-2.30 – 3.02)
Hb (g/dL)	8.13 ± 0.98	8.34 ± 0.84	10.8 ± 2.6	0.834 (0.42 – 1.88)	0.751 (0.22 – 2.17)	0.051 (0.91– 2.58)
				0.811 (0.54 – 1.07)	0.806 (-1.65 – 2.15)	0.071 (0.47 –2.38)
Reticulocyte count (%)	0.52 ± 0.81	0.71 ± 0.74	1.09 ± 0.92	0.434 (-0.18 – 2.23)	0.546 (0.79 – 1.31)	0.041 (0.95 – 2.21)
				0.372 (-0.10 – 0.2.36)	0.463 (-0.54 – 0.98)	0.039 (-0.74 – 3.22)
HCT (%)	25.01 ± 5.31	27.5 ± 3.4	33.4 ± 1.5	0.651 (0.15 – 1.87)	0.578 (-0.96 – 1.31)	0.026 (1.10 – 2.45)
				0.532 (-0.12 – 1.93)	0.429 (-0.53 – -1.32)	0.019 (-1.75 – 2.71)

Abbreviation: (1a and 1b) Significant difference between the parameters at the baseline and 4 weeks after L-glutamine therapy, (2a and 2b) Significant difference between the parameters in 4 and 8 weeks after L-glutamine therapy (3a and 3b), Significant difference between the parameters at the baseline and 8 weeks after L-glutamine therapy. No: Number; RBC: Red blood cell; Hb: Hemoglobin; HCT: Hematocrit; SD: Standard deviation, CI: confidential interval

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Discussion

In the historical background of the SCA is an inherited form of anemia in which RBCs become rigid and sticky. Sickle-shaped RBCs can lead to a slow or blocked blood flow and reduced oxygenation to parts of the body through tiny blood vessels. Periodic episodes of pain are the serious symptoms of SCA that usually involve different organs such as chest, abdomen, joints, or even bones. As several studies have shown, due to the reduction of the NAD redox potential in sickle RBCs, oxidative stress might play a significant role in the pathophysiology of pain in SCA (22, 23). Caring those who suffer from SCA may include infection prevention, blood transfusion, hydroxyurea therapy, or advanced methods such as bone marrow transplantation and gene therapy. However, due to limitations such as insufficient knowledge and appropriate donors in gene therapy and bone marrow transplantation, respectively, these progressive methods are only used for very severe cases of SCA (24, 25). In addition, it has been shown that oral L-glutamine supplementation can raise intracellular NAD metabolism and improve clinical responses in patients with SCA (26).

Based on these findings, the SCA patients in this pilot clinical trial underwent oral L-glutamine therapy for 8 weeks, and the effect of L-glutamine on pain was investigated. The mean number of pain crises over 4 weeks of L-glutamine therapy was lower than that in the period before treatment. In addition, the mean number of pain crises was significantly lower in 8 weeks after L-glutamine therapy compared to the period before the initiation of this course. As previous studies have shown, this finding might be because L-glutamine is responsible for the homeostasis of NAD and the reduced oxidative stress force in sickle RBCs (6). These findings concur with the results gained by Ilboudo et al. (27) who reported that oral L-glutamine therapy for SCA was directly correlated with clinical improvement and the reduction of pain.

Furthermore, RBC, Hb, and reticulocytes counts were found to be higher at the end of the 4-week period of L-glutamine therapy than the baseline, although the difference was not statistically significant. This difference was statistically significant at the end of the 8-week period of L-glutamine therapy. Niihara et al (17) reported an improvement in the morphology of RBCs in the second phase of their randomized trial. However, in contrast to the present study, there were no changes in the Hb or reticulocyte counts.

So far, few studies have investigated the impact of L-glutamine therapy on RBCs characteristic in SCA, but the hypothesis in this study is that increased NAD production can increase defense against oxidative stress. This hypothesis is based on the facts that L-glutamine is responsible for NAD metabolism and the NAD redox potential plays a role in sickle cell RBCs. The process of NAD production can be associated with sickle-shaped RBCs reduction and an increase in the number of normal RBCs in the blood flow. On the other hand, as it seems, due to a lack or low level of NAD in sickle RBCs, oxidative stress can have a critical role in accelerated intravascular hemolysis, which results in severe anemia in patients (22).

It is obvious that one main cause of pain in SCA is the presence of sickle RBCs in the circulation. Abnormal erythropoiesis can elevate the level of circulating sickle RBCs as well as the periodic episodes of pain crisis. In this regard, it has been shown that regular transfusion can minimize the circulating abnormal RBCs and the pain crisis via suppressing oxidative stress, particularly in the homozygote form of SCA (28, 29). In addition, hydroxyurea, alone or with blood transfusion, is frequently used to prevent metabolic stress and pain crisis in SCA (30). It has been shown, of course, that blood transfusion may be associated with alloimmunization, hemolytic reactions and

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severe clinical consequences in β°-thalassemia (31). Although we do not know how long our patients had been receiving hydroxyurea, we believe oral L-glutamine therapy could be a supplemental treatment for the SCA patients who are resistant to blood transfusion or hydroxyurea therapy.

Conclusion

In conclusion, oral L-glutamine therapy can reduce pain crisis as a common complication of SCA or sickle β°-thalassemia. In addition, the efficacy of this therapy may be recognized early by the assessment of erythropoiesis indices such as hematocrite as well as the count of normal RBCs and reticulocytes. In the present study, a series of periodic episodes of pain crisis were observed in the patients who underwent L-glutamine therapy. Thus, a decision was made to use L-glutamine therapy in optimal durations based on the severity of pain crisis. In such cases, appropriate guidelines with a sufficient level of information are needed to overcome the barriers and effectively implement the approach. The pilot clinical trial conducted in this research has some limitations. First, there were a limited number of patients; sufficient data were needed about high-risk individuals including patients with homozygote SCA and low-risk ones including patients with heterozygote forms or SCA versus sickle β°-thalassemia. Second, considering the findings of previous studies that chest pains and cardiac arrest are the possible adverse effects of L-glutamine in the long run (18) as well as the withdrawal of some patients from the study due to the exclusion criteria, such as medical intervention, blood transfusion or pregnancy, it was not possible to follow up the patients for two years. Therefore, more research with higher numbers of patients is necessary to detect the benefits of L-glutamine for pain crisis reduction in patients with SCA and sickle β°-thalassemia.

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Conflict of interest

The authors declare no conflict of interest.

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