

The GLOBE Trial: Efficacy and Safety of L-Glutamine Plus Hydroxyurea Versus Hydroxyurea Alone in Sickle Cell Anemia - A Double-Blind, Randomized Study

GLOBE Çalışması: Orak Hücreli Anemide L-Glutamin ve Hidroksiürenin Birlikte Kullanımının, Tek Başına Hidroksiüre ile Karşılaştırıldığında Etkinlik ve Güvenliliği: Çift Kör, Randomize Bir Çalışma

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Abstract

Objective: Hydroxyurea (HU) reduces complications of sickle cell anemia (SCA), but the response is variable. L-glutamine, an antioxidant that improves redox balance, is implicated in a distinct pathophysiological pathway and may provide additional clinical benefit when added to HU. We evaluated HU plus L-glutamine versus HU alone in pediatric/adolescent SCA.

Materials and Methods: In a 6-month, double-blind, placebo-controlled trial, 53 patients with HbSS or HbS/ β^0 -thalassemia were randomized to the HU + L-glutamine (n=27) or the HU + placebo (n=26) group while continuing HU at ~20 mg/kg/day. The primary endpoint was vaso-occlusive crisis (VOC) frequency; secondary endpoints included acute chest syndrome (ACS), hospitalizations, and hematological parameters. Analyses were performed for intention-to-treat with baseline-adjusted models for key outcomes.

Results: Over 6 months, the HU + L-glutamine group experienced significantly fewer VOCs (1.00±0.73 vs. 1.65±0.80; p=0.003) and ACS episodes (0.19 vs. 0.77; p=0.006). Hospitalizations declined by 40% (p=0.04). Hemoglobin (Hb) rose more in the combination arm (+0.78 vs. +0.32 g/dL; p=0.028), with larger reductions in reticulocytes (p=0.04) and greater fetal Hb increases (+6.2% vs. +1.6%; p<0.001). Adherence exceeded 80% in both arms and no serious adverse events occurred.

Öz

Amaç: Hidroksiüre (HU), orak hücreli aneminin (OHA) komplikasyonlarını azaltmaktadır; ancak tedaviye yanıt değişkenlik göstermektedir. Redoks dengesini iyileştiren bir antioksidan olan L-glutamin, farklı bir patofizyolojik yolda etkili olup, HU tedavisine eklendiğinde ek klinik yarar sağlayabilir. Bu çalışmada pediatrik/adölesan OHA hastalarında HU + L-glutamin tedavisi, tek başına HU ile karşılaştırılmıştır.

Gereç ve Yöntemler: Altı aylık, çift kör, plasebo kontrollü bu çalışmada HbSS veya HbS/ β^0 -talasemi tanılı 53 hasta, HU + L-glutamin (n=27) veya HU + plasebo (n=26) grubuna randomize edilmiş; tüm hastalarda HU tedavisine yaklaşık 20 mg/kg/gün dozunda devam edilmiştir. Birincil sonlanım noktası vazooklüzif kriz (VOK) sıklığı, ikincil sonlanım noktaları ise akut göğüs sendromu (AGS), hastaneye yatış ve hematolojik parametreler olarak belirlenmiştir. Analizler niyet edilen tedavi yaklaşımına göre yapılmış, temel sonlanımlar için başlangıç değerlerine göre düzeltilmiş modeller kullanılmıştır.

Bulgular: Altı aylık takip süresince HU + L-glutamin grubunda VOK sayısı (1,00±0,73'e karşı 1,65±0,80; p=0,003) ve AGS atakları (0,19'a karşı 0,77; p=0,006) anlamlı olarak daha düşük bulunmuştur. Hastaneye yatışlarda %40 azalma saptanmıştır (p=0,04). Kombinasyon tedavisi kolunda hemoglobin (Hb) düzeyindeki artış daha fazla olmuş (+0,78'e karşı +0,32 g/dL; p=0,028), retikülosit sayısında daha belirgin azalma (p=0,04) ve fetal Hb düzeyinde daha büyük artış (+%6,2'ye karşı +%1,6; p<0,001) izlenmiştir. Tedavi uyumu her iki grupta da %80'in üzerinde bulunmuş ve ciddi advers olay gözlenmemiştir.



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Abstract

Conclusion: Adding L-glutamine to HU significantly reduced VOCs, ACS, and hospitalizations while improving Hb and hemolysis markers, without added toxicity. The combination's efficacy likely reflects synergistic effects on oxidative stress and sickle cell pathophysiology. This well-tolerated combination may improve SCA control, but larger confirmatory trials are needed.

Keywords: Sickle cell anemia, Hydroxyurea, L-glutamine, Vaso-occlusive crisis, Pediatric hematology

Öz

Sonuç: L-glutaminin HU tedavisine eklenmesi, ek toksisite oluşturmaksızın VOK, AGS ve hastaneye yatışları anlamlı olarak azaltmış; Hb düzeyi ve hemoliz belirteçlerinde iyileşme sağlamıştır. Kombinasyon tedavisinin etkinliği, büyük olasılıkla oksidatif stres ve orak hücre patofizyolojisi üzerindeki sinerjik etkilerle açıklanabilir. İyi tolere edilen bu kombinasyon, OHA kontrolünü iyileştirebilir; ancak bulguların daha büyük doğrulayıcı çalışmalarla desteklenmesi gerekmektedir.

Anahtar Sözcükler: Orak hücreli anemi, Hidroksiüre, L-glutamin, Vazooklüzif kriz, Pediatrik hematoloji

Introduction

Sickle cell anemia (SCA) affects approximately 300,000 newborns annually, mainly in sub-Saharan Africa but also in the Middle East and South Asia. In southern Iran, the carrier rate is 1.43%, and the prevalence is 0.1% [1,2,3]. A β -globin mutation (Glu→Val) produces hemoglobin (Hb) S, leading to sickling, vaso-occlusion, hemolysis, and complications such as vaso-occlusive crisis (VOCs), acute chest syndrome (ACS), stroke, and organ damage. The disease causes substantial morbidity, premature mortality, and a high socioeconomic burden [4,5]. Hydroxyurea (HU) remains the standard therapy, increasing fetal hemoglobin (HbF) and reducing crises [6,7]. New agents such as L-glutamine, crizanlizumab, and voxelotor have expanded the treatment options for sickle cell disease (SCD). L-glutamine improves redox balance and, in a pivotal trial including HU-treated patients, reduced VOCs, hospitalizations, and ACS, but its high cost limits uptake [8,9,10,11,12,13]. Given their complementary mechanisms (HU increases HbF; L-glutamine reduces oxidative stress), combination therapy is considered safe and recommended for patients with suboptimal HU response. Inadequate response to HU alone often cannot be addressed by dose escalation due to toxicity [11,14,15]. In this study of children and adolescents with SCA on stable HU, we compared HU + L-glutamine versus HU alone to quantify its incremental benefit, hypothesizing fewer VOC/ACS events and improved hematological outcomes.

Materials and Methods

Patients and Study Design

This randomized, double-masked, placebo-controlled trial compared HU plus L-glutamine with HU plus placebo in patients with SCA (HbSS or HbS- β^0) over 6 months. All participants received standard-dose HU and either L-glutamine or a placebo. The study was approved by the Bushehr University of Medical Sciences Ethics Committee (approval ID: IR.BPUMS.REC.1400.107, date: 19.09.2021), registered with the Iranian Registry of Clinical Trials (IRCT20210715051904N1), and conducted according to CONSORT guidelines (Figure 1). Written informed parental consent was

obtained for all patients and assent was also obtained from patients aged ≥ 7 years. Medications and travel support were provided; patients in the placebo arm were later offered L-glutamine.

Eligible patients were ≥ 5 years, were confirmed to have HbSS/HbS- β^0 , had ≥ 2 severe VOCs in the prior year, and had received stable daily HU doses of about 20 mg/kg for ≥ 3 months. Key exclusion criteria included HbSC or HbS- β^+ thalassemia, central organ insufficiency, recent transfusion, investigational therapy, or prior L-glutamine usage.

Randomization and Blinding

Patients were randomized 1:1 to the HU plus L-glutamine group or the HU plus placebo group via a secure, web-based permuted-block system (fixed block size: 6) with the sequence generated by an independent statistician. Allocation concealment was ensured using opaque, sealed envelopes and centralized dispensing. L-glutamine and placebo sachets were indistinguishable in appearance, taste, and packaging, preserving the blinding of the participants, treating clinicians, investigators (including outcome assessors and analysts), and event adjudicators; only the dispensing pharmacist, who had no role in patient care, assembled sequentially numbered medication kits and held the code until database lock and trial completion.

Sample Size

Based on the sample size estimation reported by Niihara et al. [8], we estimated that 114 participants, with 57 per arm, were needed to achieve 80% power to detect a difference in VOC rate with two-sided $\alpha=0.05$ and power $(1-\beta)$ of 0.80, assuming an approximately 25%-30% reduction in VOC with add-on L-glutamine and variability consistent with the prior trial. Due to feasibility constraints at our center, we targeted 60 enrollments and ultimately randomized 53 patients, yielding about 70% post hoc power. Therefore, the study should be considered as exploratory/pragmatic, with limited power for secondary endpoints, although the primary outcome remained statistically significant with wider confidence intervals (CIs).

Intervention

L-glutamine (Endari®) was administered orally at a dose of 0.3 g/kg twice daily (maximum: 30 g per day), provided in 5 g, 10 g, or 15 g powder sachets to be mixed with food or beverages. Placebo sachets contained weight-matched inert maltodextrin powder. HU dosing was maintained at the pre-study stable dose, averaging 20 mg/kg/day, with adjustments for tolerance. Adherence was reinforced through monthly visits, weekly calls, and sachet counts. Both groups received folic acid, preventive penicillin, and occasional transfusions or analgesics.

Outcome Measures

The primary endpoint was the rate of VOCs during 6 months of treatment. A VOC was defined as an acute sickle-related pain episode requiring medical evaluation and parenteral analgesia, and ACS, sequestration, stroke, and priapism were also counted as VOC-equivalent events [14]. ACS was characterized by new pulmonary infiltrate, fever, and/or respiratory symptoms [15].

Secondary endpoints included the proportion of patients with ≥ 1 VOC, time to first VOC, number of hospitalizations for VOC/ACS, emergency department (ED) visits for sickle pain, and changes in Hb, hematocrit, reticulocyte count, and HbF from baseline to 6 months. Increases in HbF and mean corpuscular volume (MCV), along with decreases in reticulocytes and absolute neutrophil count (ANC), indicated the HU effect and adherence. Safety endpoints included the incidence of adverse events, serious adverse events, and deaths, with investigator-attributed causality.

Data Collection and Monitoring

Baseline evaluation included medical history/physical examination and laboratory testing (complete blood count with differential, reticulocyte count, Hb electrophoresis for HbA%/HbF%/HbS%, liver/renal function, lactate dehydrogenase [LDH], and infection screening, plus pregnancy testing when indicated), followed by monthly visits for 6 months to document interval events, perform targeted examinations, assess adherence (interviews and pill/sachet counts), and repeat complete blood count/reticulocytes/chemistry, with electrophoresis repeated at the end of the study. All assays were performed in the hospital's central laboratory under routine quality control standards using the HPLC Bio-Rad VARIANT II for Hb fractions (Bio-Rad, Hercules, CA, USA), Sysmex XN-series fluorescence flow cytometry for reticulocytes (Sysmex, Kobe, Japan), and Roche Cobas c501 for LDH/biochemistry (Roche, Basel, Switzerland). The inter-assay coefficients of variation were $< 3\%$ for HbF and $< 5\%$ for reticulocytes/chemistry and personnel were blinded to the allocation. Suspected VOC/ACS events were independently adjudicated by two blinded pediatric hematologist-oncologists using predefined criteria and deidentified clinical/imaging data (with a third adjudicator prespecified but not required).

Discrepancies were resolved by consensus, with a third hematologist available if needed, though not used. Chest X-rays were interpreted by radiologists blinded to treatment status, while the Data and Safety Monitoring Committee reviewed interim safety and endpoint data for any patterns.

Adherence Assessment

Adherence to study medications was monitored via pill counts and self-reports, with rates exceeding 80% in both treatment arms, indicating good compliance. Pharmacokinetic or biomarker-based assessments were not conducted. Safety was overseen by an independent Data and Safety Monitoring Committee, with no concerns raised after an interim analysis of 25 patients.

Statistical Analysis

Analyses followed intention-to-treat. Continuous variables are reported as mean \pm standard deviation (SD) or median (interquartile range) and categorical variables as number and percentage. Normality was assessed using the Shapiro-Wilk test and Q-Q plots. Between-group comparisons used t-tests or Mann-Whitney U tests for continuous data and chi-square or Fisher exact tests for categorical data; within-group differences were evaluated with paired t-tests or Wilcoxon signed-rank tests. In contrast, comparisons between groups were conducted with two-sample t-tests or Mann-Whitney tests as appropriate. Analysis of covariance (ANCOVA) models were used to enhance precision and adjust for baseline values for key outcomes, including Hb, HbF%, reticulocytes, and VOC counts, with adjustments for the baseline annualized VOC rate. The time-to-first-VOC was analyzed using Kaplan-Meier curves, with log-rank testing and a Cox model to estimate hazard ratios (HR); proportional hazards were assessed using Schoenfeld residuals. A two-sided p value of less than 0.05 was deemed significant; no multiplicity adjustment was implemented due to the study's exploratory design. Missing data constituted less than 5% and were addressed through complete-case analysis, following confirmation of missing-at-random data. All analyses were conducted using IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA), with figures produced in GraphPad Prism 9 (GraphPad Inc., La Jolla, CA, USA) and IBM SPSS Statistics.

Results

Baseline Characteristics

Between June 2019 and January 2021, 60 patients were screened for eligibility. Seven were excluded as 5 did not meet the inclusion criteria and 2 declined to participate. Fifty-three patients were randomized to the HU + L-glutamine group (n=27) or the HU + placebo group (n=26). All randomized participants completed the 6-month outcome assessments and were included in the final analysis (Figure 1). The CONSORT flow diagram in Figure 1 shows that the reasons for participant exclusion were as follows: in the L-glutamine + HU group, 3 participants were lost to follow-up,

2 withdrew consent, and 1 had a protocol violation, and in the placebo + HU group, 2 withdrew consent and 1 had a protocol violation. Despite these exclusions, all randomized participants were included in the intention-to-treat analysis.

The mean age of participants was 13.5 ± 7.4 years and nearly half (49%) were younger than 12 years. The genotype distribution was balanced between the groups (HbSS: 45%, HbS/ β^0 -thalassemia: 55%), with no HbSC or other variants. During the trial, all patients received a nearly constant dose of HU (20 mg/kg/day), with an average dose of 19.8 mg/kg (SD: 2.5). Both groups had evidence of mild-to-moderate baseline anemia and hemolysis, typical for SCD. Clinical and laboratory characteristics are detailed in Table 1. The baseline characteristics were essentially the same for both treatment groups. For example, Hb levels were approximately 9.5 g/dL, HbF levels were approximately 15% (slightly higher in the L-glutamine group), reticulocyte counts were approximately $260 \times 10^3/\mu\text{L}$, and LDH levels were approximately 580 U/L. The dosages of HU and folate were also similar between the groups. The HU + L-glutamine group showed a slightly higher pre-enrollment VOC burden (mean: 2.6 vs. 2.0/year; median: 2 for both; $p=0.10$). Baseline ACS history and organ-damage markers were similar (ACS uncommon; median: 0), which means that randomization worked even if there were some minor differences.

Primary Outcome: Pain Crisis Frequency

The 6-month trial indicated that patients receiving HU + L-glutamine had significantly fewer VOCs than those receiving

only HU (1.00 ± 0.73 vs. 1.65 ± 0.80 crises per patient; 95% CI: 0.23-1.07; $p=0.003$), reflecting a 40% reduction. The HU + L-glutamine group demonstrated a decrease of 0.65 crises per patient relative to the HU-only group (95% CI: 0.23 to 1.07 fewer). This corresponds to a similar 40% relative reduction from a baseline of 1.65 crises per patient (Figure 2). More patients were crisis-free with L-glutamine (7/27, 26%) than with the placebo (2/26, 8%). Both groups improved from baseline, but the reduction was greater with L-glutamine ($p<0.001$ vs. $p=0.08$). ANCOVA adjusted for baseline VOC rate showed a significant benefit for HU + L-glutamine ($p<0.001$). Crisis-free survival was more prolonged (120 vs. 80 days), and time-to-first VOC favored combination therapy with a HR of approximately 0.58, but these time-to-event differences were not statistically significant ($p \approx 0.10-0.11$), likely due to limited power. Overall, VOC reduction and more crisis-free patients support the additive effect of L-glutamine.

Secondary Outcome: Acute Chest Syndrome

ACS was less frequent with HU + L-glutamine (5/27 [19%] vs. 14/26 [54%]; risk ratio [RR] ≈ 0.35 ; $p=0.055$), with fewer episodes per patient (0.19 vs. 0.77; $p=0.006$) (Figure 3). Although both incidence proportions and event rates indicated fewer ACS episodes in the L-glutamine + HU group, the small number of events means that these findings should be interpreted with caution and are considered hypothesis-generating due to the limited statistical power of this exploratory study.

Table 1. Baseline characteristics of the study population.

Variable	HU + placebo (n=26)	HU + L-glutamine (n=27)	p
Demographics			
Age, years (mean \pm SD)	13.6 \pm 7.2	13.5 \pm 7.4	0.84
Male sex, n (%)	12 (46%)	13 (48%)	0.88
Female sex, n (%)	14 (54%)	14 (52%)	0.88
HbSS genotype, n (%)	12 (46%)	12 (44%)	0.89
HbS/ β^0 -thalassemia genotype, n (%)	14 (54%)	15 (56%)	0.90
Hemoglobin, g/dL (mean \pm SD)	9.62 \pm 0.98	9.11 \pm 0.97	0.06
HbF, % (mean \pm SD)	13.75 \pm 5.41	16.59 \pm 5.38	0.06
Absolute reticulocyte count, $\times 10^3/\mu\text{L}$ (mean \pm SD)	107.3 \pm 28.9	129.3 \pm 32.9	0.012*
MCV, fL (mean \pm SD)	95.1 \pm 3.5	95.0 \pm 3.2	0.81
ANC, $\times 10^9/\text{L}$ (mean \pm SD)	5.48 \pm 1.30	5.20 \pm 1.25	0.67
VOCs in past 6 months, median [IQR]	2 [1-3]	3 [2-4]	0.020*
ACS history, n (%)	13 (50%)	14 (52%)	0.49
HU dose (mg/kg/day, mean \pm SD)	19.8 \pm 2.5	19.8 \pm 2.5	-

*: $p<0.05$.

Values are presented as mean \pm SD, median [IQR], or n (%). The p values compare groups at baseline using the Student t-test or Mann-Whitney U test for continuous variables and the chi-square or Fisher exact test for categorical variables as appropriate.

SD: Standard deviation; IQR: interquartile range; HU: hydroxyurea; HbF: fetal hemoglobin; MCV: mean corpuscular volume; ANC: absolute neutrophil count; VOC: vaso-occlusive crisis; ACS: acute chest syndrome.

Hospitalizations and Hematological Parameters

The HU + L-glutamine group had fewer sickle cell-related hospitalizations than the placebo group (44% vs. 69%, mean of 0.46 vs. 1.0 admissions, $p=0.04$), with shorter total hospital days (55 vs. 88). Combination therapy led to a modest but significant Hb increase (+0.46 g/dL; 95% CI: +0.05 to +0.87; $p=0.028$; +0.78 vs. +0.32 g/dL), with a higher proportion of patients achieving Hb of ≥ 10 g/dL (59% vs. 42%). Reticulocyte counts declined more markedly (-19 vs. $-8 \times 10^3/\mu\text{L}$, $p=0.04$), accompanied by lower LDH trends, indicating reduced hemolysis (Figures 4A and 4B). HbF rose significantly with HU + L-glutamine (+6.2% vs. +1.6%, $p<0.001$), reaching 22.8% vs. 15.4% at 6 months, with an absolute increase of +4.6% (95% CI: +2.0% to +7.2%) indicating a statistically robust effect (Figure 5). Although L-glutamine does not directly induce HbF, its combination with HU likely enhanced erythropoiesis and redox stability. HU adherence exceeded 80% in both groups, minimizing compliance bias. Non-admission ED visits were less frequent with HU + L-glutamine (17 vs. 33), consistent with fewer VOCs and reduced acute healthcare utilization.

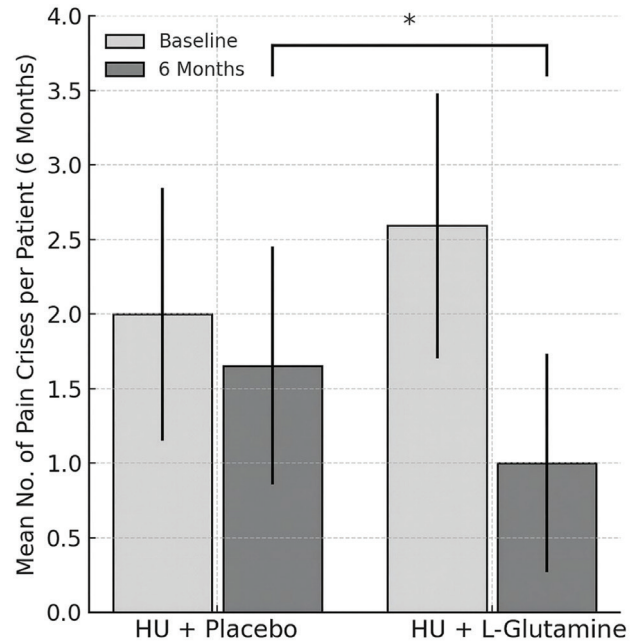


Figure 2. Mean number of vaso-occlusive crises (VOCs) per patient at baseline and after 6 months of treatment in each arm. The hydroxyurea (HU) + L-glutamine group’s VOC rate declined from 2.6 (baseline) to 1.0 at 6 months, versus a change from 2.0 to 1.65 in the HU + placebo group ($p=0.003$ between groups).

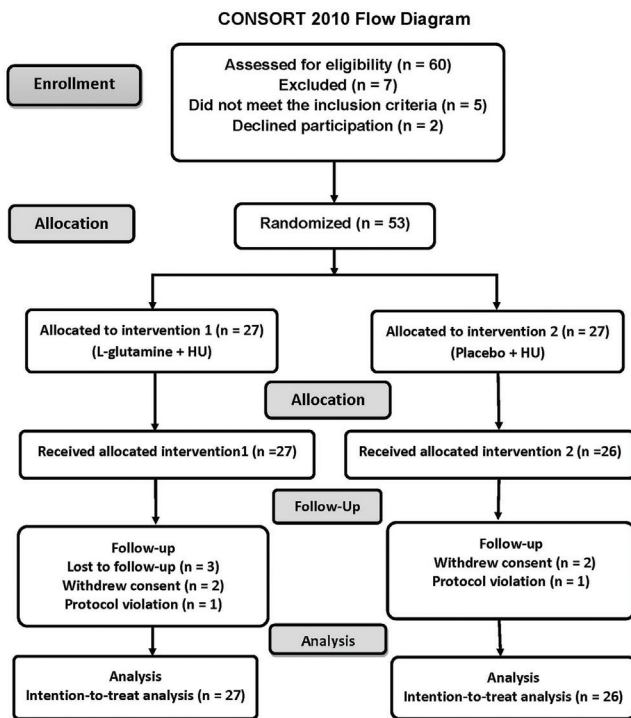


Figure 1. CONSORT flow diagram of the trial. A total of 60 patients were screened for eligibility. Seven were excluded (5 did not meet the inclusion criteria and 2 declined participation), leaving 53 participants who were randomized 1:1. In the L-glutamine + hydroxyurea (HU) group, 6 participants did not complete follow-up: 3 were lost to follow-up, 2 withdrew consent, and 1 had a protocol violation. In the placebo + HU group, 3 participants did not complete follow-up: 2 withdrew consent and 1 had a protocol violation. Despite these exclusions, all randomized participants were included in the final intention-to-treat analysis.

Safety and Adherence

Adherence to HU and L-glutamine exceeded 80%, confirmed by pill counts and follow-up, with stable MCV/ANC and expected HU-related changes (increased HbF, reduced reticulocytes) supporting comparable HU exposure and objective adherence. L-glutamine was well tolerated, with no serious adverse events or discontinuations. Mild, transient gastrointestinal symptoms occurred in ~15% of the cases and laboratory parameters remained stable, supporting the safety and feasibility of L-glutamine as an adjunct to HU.

Discussion

Principal Findings and Interpretation

In the GLOBE study, adding oral L-glutamine to HU improved pediatric/adolescent SCA outcomes over 6 months, reducing VOCs, ACS, and hospitalizations and modestly improving Hb and hemolysis markers (reticulocytes, LDH). Although L-glutamine does not directly induce HbF production, the greater HbF rise observed with combination therapy likely reflects indirect effects such as an improved redox environment and erythropoiesis. The observed increase in HbF in the HU + L-glutamine group in the present study is intriguing and unexpected, as L-glutamine is not known to directly induce HbF production. Although baseline adjustments were made to account for potential confounders, alternative explanations may also account for these findings.

For example, improved adherence to HU therapy due to enhanced patient support or a potential regression to the mean effect could have contributed to the HbF increase. Further studies are needed to clarify the biological mechanisms underlying this effect [16,17]. Overall, L-glutamine may potentiate HU's benefits by mitigating oxidative stress and improving HU responsiveness and adherence, thereby sustaining red cell integrity and clinical stability.

Our results corroborate and extend the findings of the phase 3 trial conducted by Niihara et al. [8], which showed reduced VOCs with L-glutamine. Whereas Niihara et al. [8] reported a 25% lower median annual pain-crisis rate, we observed a 40% relative reduction in VOC over 6 months in HU-treated patients, supporting an additive benefit. The primary endpoint remains robust: baseline-adjusted analyses continue to show significantly lower VOC rates with HU + L-glutamine, despite baseline imbalance. Unlike Niihara et al. [8], we observed improvements in Hb and HbF, potentially reflecting our younger, uniformly HU-treated cohort and intensive adherence monitoring. These findings are consistent with phase 2 data from Ebeid et al. [18], who showed fewer acute SCD complications with L-glutamine.

Our trial reaffirmed the safety of the combination, showing no increase in adverse events or laboratory toxicity with L-glutamine, consistent with recent studies [18,19]. Gastrointestinal symptoms were mild with no discontinuations, and comparable neutrophil/cytopenia profiles suggest that L-glutamine does not exacerbate HU myelosuppression. High adherence likely reflected good tolerability and easy administration, supporting the practicality

of combination therapy; adherence is reinforced by education, monitoring, access, family support, and strong provider relationships [9,20,21].

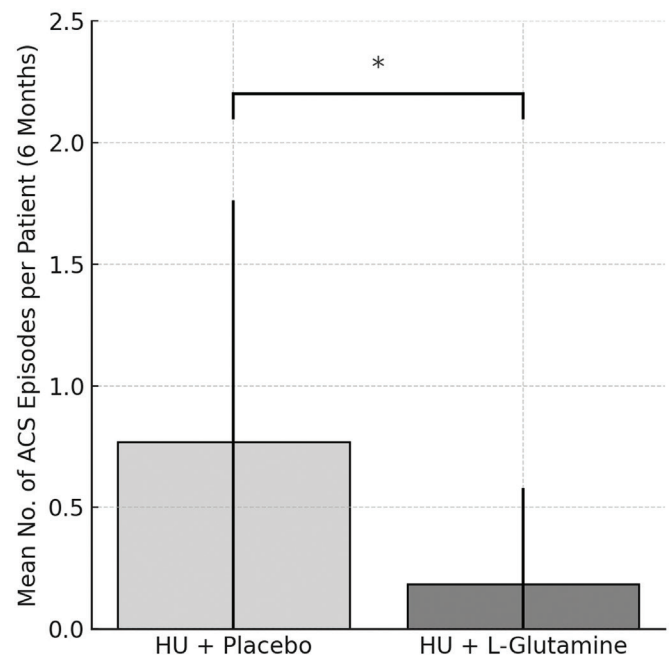


Figure 3. Mean number of acute chest syndrome (ACS) events per patient over 6 months in each group. The hydroxyurea (HU) + L-glutamine arm averaged 0.19 ACS episodes vs. 0.77 in the HU + placebo group ($p=0.006$). The incidence proportions were 19% vs. 54%. The ACS analysis had limited statistical power due to the small number of events, which should be considered when interpreting the results.

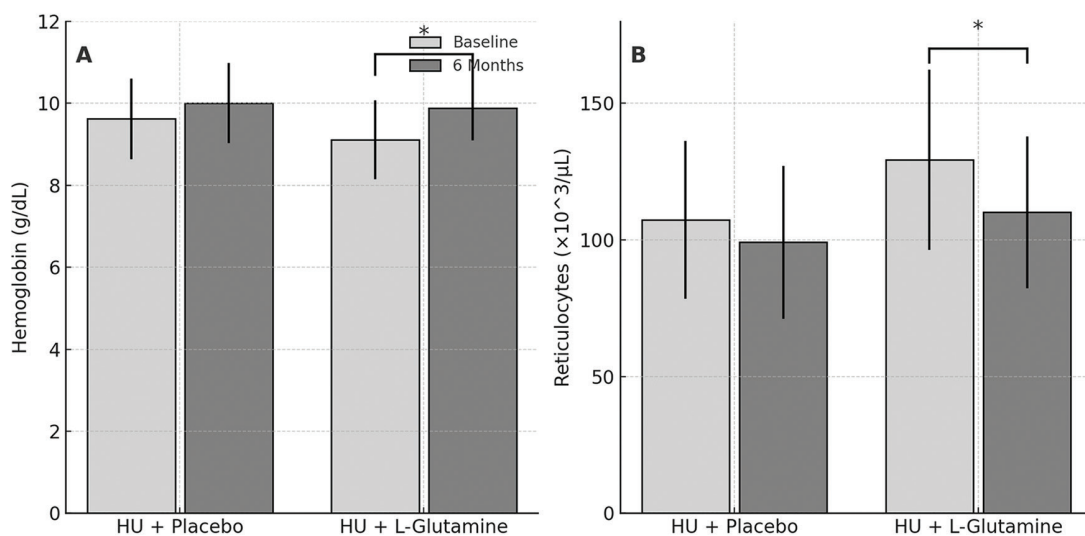


Figure 4. A) Mean hemoglobin levels at baseline and 6 months for both groups. The hydroxyurea (HU) + L-glutamine group showed a significant increase in hemoglobin from ~ 9.4 to 10.2 g/dL on average, whereas the HU-only group showed a more minor increase from ~ 9.5 to 9.8 g/dL; error bars indicate standard deviations. B) Mean absolute reticulocyte counts at baseline and 6 months. The HU + L-glutamine group had a larger decrease in reticulocyte count, indicating reduced hemolysis, compared to minimal change in the HU-only group; error bars indicate standard deviations.

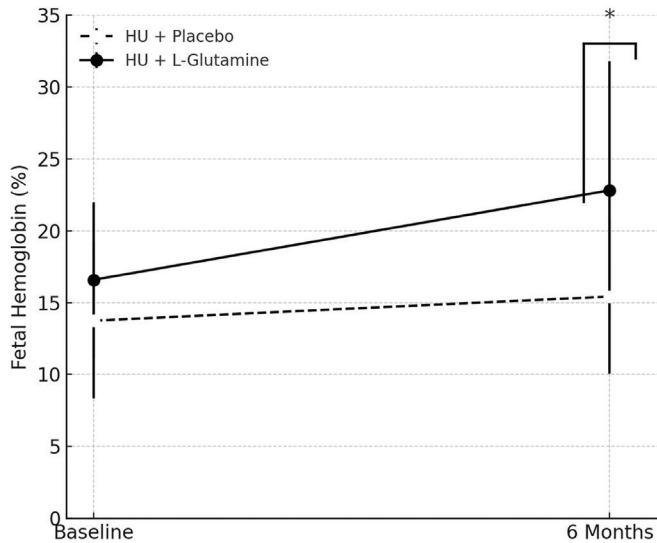


Figure 5. HbF levels at baseline and after 6 months in both groups. The hydroxyurea (HU) + L-glutamine group increased from 16.6% \pm standard deviation to 22.8% ($p < 0.001$), whereas the HU + placebo group went from 13.8% to 15.4% (no significant change). Error bars show standard deviations.

HbF: Fetal hemoglobin.

Mechanistic Implications

The findings indicate an oxidative-stress mechanism: L-glutamine, as a nicotinamide adenine dinucleotide precursor, enhances redox defenses (e.g., glutathione), reduces hemolysis and endothelial adhesion, and may improve vascular function and nitric oxide bioavailability. This is consistent with improved Hb and reticulocyte levels, as well as fewer crises, thereby reinforcing the need for multimodal therapy beyond HU-driven Hb F induction [12,16,17,18,19,22,23].

Study Limitations

Due to feasibility constraints, we targeted 60 participants and randomized 53, with approximately 70% post hoc power. This single-center study with 6-month follow-up should therefore be viewed as a pragmatic, exploratory trial with wider CIs and limited power for some secondary endpoints such as ACS. Nonetheless, the primary outcome remained statistically significant, and prespecified baseline-adjusted analyses, together with consistent, clinically meaningful improvements in VOCs, hospitalizations, and hematological indices, support the robustness and clinical relevance of the findings and warrant confirmation in larger multicenter studies.

Conclusion

This randomized trial shows that adding L-glutamine to HU significantly reduces VOCs and ACS while improving Hb and hematological indices without added toxicity. The combination's efficacy likely reflects synergistic effects on redox balance and

sickle cell pathophysiology, with excellent adherence and tolerability.

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Ethics

Ethics Committee Approval: The study was approved by the Bushehr University of Medical Sciences Ethics Committee (approval ID: IR.BPUMS.REC.1400.107, date: 19.09.2021).

Informed Consent: Written informed consent was obtained from all parents/guardians, and assent was also obtained from children ≥ 7 years.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.S., M.Mo., M.R.; Concept: N.S., M.R.; Design: N.S., M.R.; Data Collection or Processing: M.Mo., N.S., M.Ma., B.A., M.R.; Analysis or Interpretation: M.Ma., B.A.; Literature Search: M.Ma., N.S.; Writing: N.S., M.Ma., B.A., M.Mo., M.R.

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