original reports

Randomized, Parallel Group, Open-Label Bioequivalence Trial of Intramuscular Pegaspargase in Patients With Relapsed Acute Lymphoblastic Leukemia

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abstract

PURPOSE Pegylated asparaginase is comparatively safer than native asparaginase in the management of acute lymphoblastic leukemia (ALL). However, the high price and nonavailability in low- and middle-income countries limits its use. In 2014, the first generic of pegaspargase (Hamsyl) was approved in India for use as a second-line treatment option for ALL. The aim of this study was to assess whether the generic pegaspargase (the test product) was bioequivalent with the reference product (Oncaspar).

PATIENTS AND METHODS This study was an open-label, parallel-group, comparative pharmacokinetic study in pediatric patients with relapsed ALL receiving their first dose (1,000 IU/m^2) of pegaspargase administered intramuscularly. Patients were randomly assigned 1-to-1 to either the test or the reference product. The 2 formulations were considered equivalent if the 90% CIs for area under the plasma asparaginase activity–time curve (AUC_{0-t}) geometric mean test-to-reference ratio was within 75% to 133%.

RESULTS Twenty-nine patients (6-18 years of age) were enrolled in this study, of whom 24 completed the study criteria and were considered for safety analysis (5 patients were ineligible for the assessment). Three patients were excluded from analysis, because of presence of anti-asparaginase antibodies, leaving 21 patients who were considered for bioequivalence pharmacokinetics data. The point estimate of AUC_{0-t} for the test-to-reference ratio was 95.05 (90% CI, 75.07% to 120.33%). Maximum plasma concentration, trough concentrations (day 14), half-life, volume of distribution, drug clearance, and changes in the asparagine and glutamine levels were not significantly different between products. Adverse events were comparable in both groups.

CONCLUSION Generic and reference pegaspargase had equivalent pharmacokinetics with comparable safety. This could be a safe and cost-effective alternative for patients with ALL, especially in low- and middle-income countries.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant conversion of rapidly growing lymphoid progenitor cells.[1,2] Patients with ALL in higher-income countries have better survival rates (> 80%) than do those in low- and middle-income countries (LMICs), for whom ALL survival rates are lower and range from 36% to 53%, which could be due to limitations in health care, differences in general health, and maybe the biology of ALL.[3,4] Furthermore, approximately 15% to 20% of pediatric ALL cases relapse after first complete remission, and these cases are usually treated with either chemotherapy and/or hematopoietic stem cell transplant.[1,5] In all the ALL treatment protocols, L-asparaginase is a key drug of combination chemotherapy regimens.[6-8] Three types of asparaginases are approved for ALL: native *Escherichia coli* L-asparaginase, pegylated asparaginase (pegaspargase), and native *Erwinia* L-asparaginase (isolated from *Erwinia chrysanthemi*).[6] Currently, in India, native L-asparaginase is approved for use in first-line of treatment of ALL; however, it is a matter of concern that, compared with the established norms, available generic formulations of L-asparaginase in India are found to have subtherapeutic asparaginase activity in the patients.[9]

L-asparaginase efficacy is limited by silent immunity and acute allergic reactions,[8] which necessitate switching to *Erwinia* L-asparaginase. To overcome these limitations, pegylated formulations of *Escherichia coli* L-asparaginase were developed and approved by the

ASSOCIATED CONTENT

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

To study whether the generic pegaspargase (Hamsyl) is safe and equivalent to innovator pegaspargase (Oncaspar).

Knowledge Generated

Two formulations of pegaspargase are bioequivalent and the generic could be used as an alternative to the innovator L-asparaginase in relapsed acute lymphoblastic leukemia (ALL) patients. Both the formulations have the comparable safety profile. Both the formulations are able to produce the levels of asparaginase levels more than the recommended 100 IU/L even at day 14 of pegaspargase administration.

Relevance

Generic pegaspargase could be cost-effective alternative for patients with ALL especially in low- and middle-income countries because many patients there cannot afford the high cost of the innovator pegaspargase.

US Food and Drug Administration in 1994.[2] Pegylated forms of L-asparaginases have L-asparaginase bound to monomethoxy polyethylene glycol (PEG) with a succinimidyl succinate linker (pegaspargase with succinimidyl succinate–PEG) or a succinimidyl carbamate linker (calaspargase pegol with succinimidyl carbamate–PEG).[10-13] Pegylation causes a 5-fold increase in half-life of the drug, resulting in longer drug activity.[10-14] Innovator pegaspargase is less affordable for patients with middle to low incomes and is not readily available to be imported on a named-patient basis,[15] which limits needy patients' access to this drug. Introduction of generic pegaspargase can make it affordable and more available in LMICs, which is evident in the case of other biosimilars approved in the past. There is a history of lowered pricing of biosimilars compared with reference biologic products (eg, 36% difference for etanercept, 39% for rituximab, and 31% for infliximab, whereas at retail level, the differences are 11%, 86%, and 143%, respectively.[16] In 2014, Gennova Biopharmaceuticals Ltd (Pune, India) received approval for manufacturing and marketing of a generic version of pegaspargase under the brand name of Hamsyl. Here, we report a bioequivalence study of Hamsyl, hereafter called the test product, and a reference product, Oncaspar; Servier Pharmaceuticals, Boston, MA), in patients in India with relapsed ALL.

PATIENTS AND METHODS

Study Design, Patients and Setting

This study was an open-label, randomized, parallel-group, comparative pharmacokinetic study of 2 formulations of pegaspargase in relapsed pediatric cases of ALL. The study protocol and its amendments were approved by the subject expert committee and institutional ethics committee of our hospital and the Drug Controller General of India (DCGI). The study was prospectively registered in the Clinical Trials Registry–India portal (reference no. CTRI/2016/02/006589) and conducted in accordance with the Good Clinical Practice guidelines and principles enunciated in the Declaration of Helsinki.

Patients with relapsed ALL, 6 to 18 years of age, were included in the study. Written informed consent was obtained from the parents or legal guardian. Children ≥ 7 years of age also signed the assent form. Patient recruitment in the study was based on the presence of blasts in the bone marrow that were myeloperoxidase negative and terminal deoxynucleotidyl transferase positive and/or expressed an ALL immunophenotype with monoclonal antibodies directed against precursor B- or T-cell lineage. Children with a history of allergy, thrombosis, or pancreatitis due to L-asparaginase were excluded from the study. Patients with ALL FAB L3 type and blast crisis of chronic myelogenous leukemia and HIV-positive patients were excluded from the study. Patients with serum levels of bilirubin ≥ 2 mg/dL and creatinine ≥ 2 mg/dL also were not included in the study.

Treatment Regimen and Sampling Technique

Pegaspargase was administered as a part of standard ALL treatment regimen. Initially, patients were enrolled in the modified Children's Oncology Group (COG) ALL treatment protocol (Data Supplement). The induction regimen of the protocol comprised dexamethasone, doxorubicin, vincristine, bortezomib, arabinoside, and triple intrathecal therapy. However, this regimen was not well tolerated by most patients, so the treatment protocol was amended to follow the modified St Jude treatment protocol (Data Supplement). The induction regimen of this protocol comprised prednisolone, vincristine, daunorubicin, arabinoside, etoposide, and triple intrathecal therapy. In both regimens, the pegaspargase dosing algorithm was same and patients were randomly assigned 1:1 to receive intramuscularly administered pegaspargase 1,000 IU/m^2, either the test product or reference product. Both formulations contained pegaspargase 3,750 IU/5 mL.

Primary End-Point Evaluation

The primary end point of this study was to compare the area under the L-asparaginase activity–time curve (AUC_{0-t}) after administration of the 2 formulations. Blood samples were collected from patients to determine the plasma

asparaginase activity before administration of pegaspargase and again at 1, 6, 24, 48, 120, 168, and 312 hours after the administration of the first pegaspargase dose.

Secondary End-Point Evaluations

Secondary end points were to compare immunogenicity, grade 3/4 nonhematological adverse reactions and the change in the levels of amino acids L-asparagine, L-aspartic acid, L-glutamine, and L-glutamic acid after the administration of pegaspargase.

Blood samples were collected on day 1 (before the administration of first dose of pegaspargase) and on day 14 for assessing the presence of anti-asparaginase antibodies and for assessing the changes in the levels of aforementioned plasma amino acids after pegaspargase administration. Plasma was immediately separated by centrifugation and stored at –80°C until further analysis.

Bioanalysis

Plasma L-asparaginase enzyme activity was measured by spectrophotometric method as reported by Sankaran et al.[9] The method is based on the principle that L-asparaginase in the patient's plasma sample hydrolyses aspartic acid β-hydroxamate to L-aspartic acid and hydroxylamine, which condenses with 8-hydroxyquinoline and oxidizes to indoxine, forming a colored compound. Compound intensity was determined spectrophotometrically at 710 nm. This assay has sensitivity to measure asparaginase activity as low as 0.002 IU/mL (calibration range, 0.002-2 IU/mL).

Immunogenicity Evaluation

To assess immunogenicity, an anti–L-asparaginase antibody assay was performed using the Human Asparaginase Antibody (Anti-ASP) ELISA Kit (MBS109077; MyBioSource, San Diego, CA), which is a ready-to-use microwell, strip-plate ELISA. L-asparagine, L-aspartic acid, L-glutamine and L-glutamic acid were measured using a validated method on liquid chromatography–tandem mass spectrometry (QTRAP 4500; AB Sciex, Framingham, MA).

Safety Laboratory Evaluation

Adverse events (AEs) observed during the study were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. Causality assessment of AEs were assessed using the Naranjo algorithm.[17] Requisite safety laboratory investigations (CBC count, and levels of glucose, bilirubin, liver enzymes, creatinine, electrolytes, fibrinogen, and antithrombin III) were performed once weekly and also as required for disease management.

Statistical Analysis

Bioequivalence assessment of the test and reference products was determined at 90% CIs for the logarithmic transformed data of $AUC_{0\text{-}t}$ with a predefined acceptable equivalence range within the limits of 75% to 133%. The primary end-point analysis was conducted per protocol approach. Other pharmacokinetic parameters, including maximum observed concentration (C_{max}), time to maximum observed concentration (t_{max}), terminal half-life, clearance, and volume of distribution, were compared between the 2 groups using an independent *t* test. Relative changes in the plasma levels of L-asparagine, L-glutamine, L-aspartic acid and L-glutamic acid (from baseline to day 14) of patients who completed all study-related activities (n = 12 in each arm) were evaluated using the Wilcoxon rank-sum method. Presence of antibodies in the patients enrolled in the 2 groups was compared using the Fisher exact *t* test.

RESULTS

Patient Characteristics and Disposition

A total of 29 patients (reference arm [n = 15]; test arm [n = 14]) were enrolled in the study from February 2016 to December 2017. Of the 29 patients, the first 8 patients (n = 4 in each arm) were treated following the modified COG protocol of ALL treatment, and the remaining patients were treated following the modified St Jude's stage III/IV ALL induction protocol (reference arm [n = 11]; test arm [n = 10]). Patients' baseline demographic data are presented in Table 1. Five of the 29 patients had to be replaced (reference arm [n = 3]; test arm [n = 2]) because of incomplete pharmacokinetic blood sampling, leaving a total of 24 patients eligible for study evaluation. Of these, 21 were considered for pharmacokinetic evaluation.

Primary End Point

Anti–L-asparaginase antibodies were present in 58.6% of patients before the administration of pegaspargase. Of these, 3 patients (reference arm [n = 1]; test arm [n = 2]) had consistently high levels of antibodies during the course of their treatment. Anti–L-asparaginase antibodies are known to affect the pharmacokinetics of pegaspargase; therefore, these patients were excluded from the primary analysis. Hence, data of 10 patients in the test arm and 11 patients in the reference arm were included in the final analysis for bioequivalence.

The primary objective of equivalence in terms of pairwise comparisons of the $AUC_{0\text{-}t}$ ratio of geometric means between the test and reference products was established. The test product had a similar kinetic time profile as the reference drug. The point estimate of $AUC_{0\text{-}t}$ for the test-to-reference ratio was 95.05% (90% CI, 75.07% to 120.33%), which was contained within the predefined acceptance range of equivalence of 75% to 133%, thus fulfilling the primary objective of this study (Table 2). Pegaspargase pharmacokinetics after the first dose were plotted with the geometric means of plasma L-asparaginase activity versus time (Fig 1). There was no statistical difference between the 2 groups for C_{max}, half-life, total clearance, and volume of distribution of asparaginase (Table 3). Mean trough levels were > 100 IU/L in both arms (117.6 ± 37.66 IU/L *v* 113.57 ± 50.24 IU/L in test *v* reference

TABLE 1. Baseline Characteristics of Patients

Baseline Characteristic	All Patients (N = 29)		Test Arm (n = 14)		Reference Arm (n = 15)	
	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Sex, No.						
Male	19		6		13	
Female	10		8		2	
Age, years	9.7	2.7	10.43	3.01	9.0	2.30
Weight, kg	30.1	12.4	36.09	14.19	26.15	8.41
Height, cm	135.3	17.6	140.43	19.79	130.53	14.35
Treatment protocol, No.						
Modified COG	8		4		4	
Modified St Jude[a]	21		10		11	

Abbreviations: COG, Children's Oncology Group; SD, standard deviation.
[a]St Jude stage III/IV acute lymphoblastic leukemia induction protocol.

arms). Figure 2 shows the distribution of trough levels in the 2 groups.

Secondary End Points

Levels of amino acids. After administration of pegaspargase, there was a significant decrease in the levels of serum L-asparagine from baseline in both the treatment arms (Fig 3), but the same was not observed for L-glutamine (Fig 4). However, there was no statistically significant difference between arms in the median reduction in L-asparagine (test *v* reference arms, respectively: –94.18% *v* –94.55%; interquartile range [IQR], –95.49% to –79.41% *v* –95.72% to –92.44%) and L-glutamine levels (test *v* reference arms, respectively: –20.48% *v* –24.39%; IQR, –35.86% to –11.44% *v* –51.86% to –6.01%).

Antibody levels. Because this study was done with patients with relapsed ALL, all had been treated previously with L-asparaginase, and anti-asparaginase antibodies were expected in some patients. More than half (58.6%) of the patients had anti-asparaginase antibodies at baseline; however, none of them developed any allergic reactions to pegaspargase. In 3 of these patients (test arm [n = 2]; reference arm [n= 1]; Fig 5), antibody titers increased by day 14 and stayed high. Apart from pre-existing antibodies, new antibody formation was not observed with either formulation during the course of treatment.

Safety assessments. None of the patients in either group developed any allergic reactions to pegaspargase. Of the 24 eligible for safety assessments, 5 in ref arm, 7 in test arm experienced grade 3/4 nonhematological AEs necessitating hospitalization (Table 4). Of these, fever with associated neutropenia was more common. However, no grade 3/4 hematologic AE was warranting drug withdrawal was noted during the study. A total of 71 AEs of any grade developed in the 29 patients (Data Supplement). None of these AEs could be attributed to the study drugs except for 1 case of pancreatitis in the test arm. There were 5 deaths reported in the study (test arm [n = 3]; reference arm [n = 2]).

DISCUSSION

In this randomized, open-label, parallel group bioequivalence study of pegaspargase formulations in patients with relapsed ALL, bioequivalence was established between the pegaspargase formulation, Hamsyl, and the reference formulation, Oncaspar. L-Asparaginase is one of the key drugs in ALL combination chemotherapy protocols and, recently, pegaspargase has been preferred to conventional L-asparaginase formulation because of the decreased incidence of allergies and prolonged half-life. It has now become the drug of choice globally for first-line treatment of pediatric ALL.[2] However, because of the high price and nonavailability of innovator drugs in India, biopharmaceutical companies have started developing generic pegaspargase. Considering the unmet need in India for pegaspargase for patients with ALL, the DCGI has approved Hamsyl with a recommendation to conduct human bioequivalence studies.

High interindividual variability in asparaginase activity has been reported in patients with ALL treated with asparaginase; considering that, bioequivalence acceptance limits were kept between 75% and 133% rather than the conventional limits of 80% to 125%.[18] The 2 formulations were bioequivalent with respect to the predefined acceptance criteria for AUC_{0-t}. Other pharmacokinetic parameters of pegaspargase—volume of distribution, clearance, and half-life—were comparable between test and reference arms. Also, the 2 formulations were comparable even in terms of pharmacodynamic response (eg, L-asparagine depletion). Interestingly, 2 patients in test arm and 1 patient in reference arm did not achieve asparagine depletion, but the reason could not be ascertained because these patients had sufficient asparaginase levels even at day 14. In pegaspargase, multiple molecules of 5-kDa PEG are attached to L-asparaginase.[19] It is

TABLE 2. Bioequivalence Criteria of Plasma Asparaginase Activity of the Test and Reference Drugs

Parameter	Geometric Least Square Mean		90% CI	T/R Ratio (%)	Power (%)	Interpatient CV (%)
	Test (n = 10)	Reference (n = 11)				
Ln AUC_{0-t} (hour × IU/L)	74,457.3708	78,337.8720	75.07 to 120.33	95.05	46.3	32.0

Abbreviations: AUC_{0-t}, area under the activity-time profile curve from time 0 extrapolated to time t; CV, coefficient of variation; Ln, natural log; R, reference; T, test.

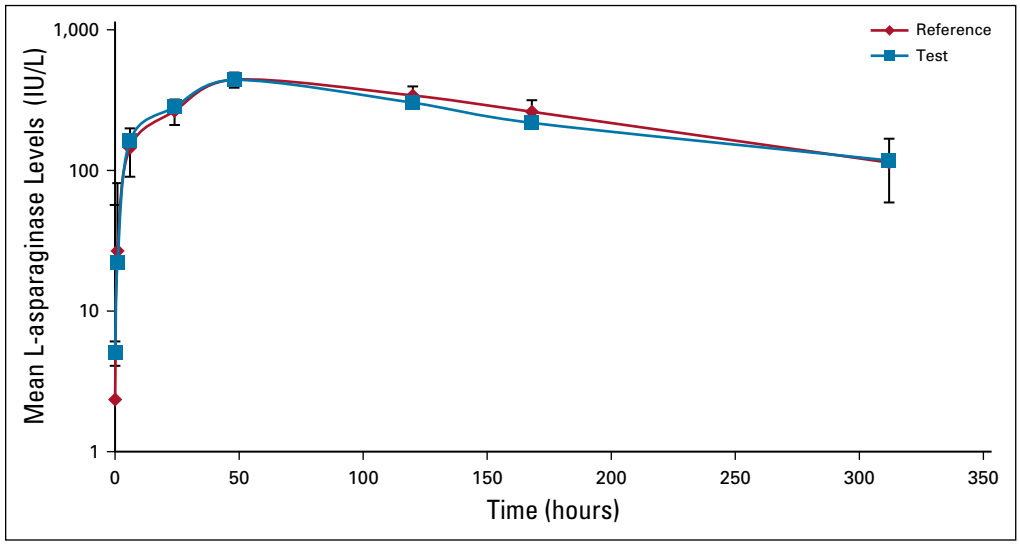

FIG 1. Mean ± standard deviation of L-asparaginase plasma activity–time profiles in the test and reference arms.

critical to have a similar number of PEG molecules on generic preparations, as well. If not, then the pharmacokinetic may differ significantly. Physicochemical characterization can be used for quality analysis and comparison. However, clinical assessment is also equally important. The similarity in pharmacokinetic profile of the 2 formulations underlines the similarity in bioavailability of the test and reference formulations.

Pegylation prolonged the half-life of L-asparaginase and there was no significant difference in plasma half-life between the 2 formulations (Data Supplement). Asselin et al[12] also observed in their study that the elimination half-life of pegaspargase was 5.73 days in anti–L-asparaginase antibody-negative patients.

The t_{max} for both the formulations was achieved by 48 hours; this parameter is discrete and is dependent on fixed time and not on continuous determination throughout the sample evaluation period. Therefore, comparing it with other parameters that characterize absorption and elimination rates is of little value.[20]

L-asparaginases have variable amount of glutaminase activity as a secondary activity, and whether the glutaminase activity has any clinical relevance is debatable.[21,22] Recent studies by Parmentier et al[23] and Chan et al[24] have shown that glutaminase activity is required along with L-asparaginase activity for the effective management of ALL. Many of the toxic adverse effects of L-asparaginase therapy previously were attributed to the L-glutaminase activity.[21,25] Conversely, 90% of L-glutamine needs to be deaminated to achieve a beneficial effect of optimal L-asparagine depletion.[25] L-Glutamine levels in serum are high compared with L-asparagine levels, requiring toxic doses of L-asparaginases to produce the required L-glutamine depletion.[26] In our study, we observed that L-glutamine levels declined by only 20% with both the formulations. In both groups, there was significant increase in the plasma

TABLE 3. Pharmacokinetic Parameters of the 2 Formulations

	Test Arm (n = 10)			Reference Arm (n = 11)		
Parameter	**Mean**	**SD (±)**	**CV**	**Mean**	**SD (±)**	**CV**
$AUC_{0\text{-}t}$ (hours × IU/L)	76,531.123	17,029.900	22.252	82,352.672	24,522.753	29.778
C_{max}, IU/L	440.02	106.00	24.090	442.05	147.86	33.450
t_{max}, hours	48	0	0	54.55	21.71	39.80
$t_{1/2}$, hours	155.28	47.31	30.47	138.91	58.58	42.18
Vd, L	8.469	3.414	40.314	7.758	4.934	63
Cl, L/h	0.038	0.011	28.093	0.038	0.012	31.466

NOTE. The *P* values comparing the 2 arms for each parameter were not statistically significant.

Abbreviations: $AUC_{0\text{-}t}$, area under the activity-time profile curve from time 0 extrapolated to time t; Cl, clearance; C_{max}, maximum observed concentration; CV, coefficient of variation; SD, standard deviation; t_{max}, time to maximum observed concentration; $t_{1/2}$, terminal half-life; Vd, volume of distribution.

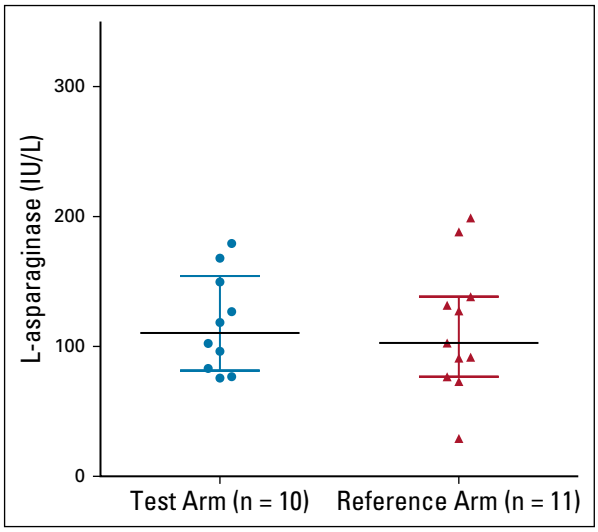

FIG 2. The L-asparaginase trough levels of individual patients in the test and reference arms.

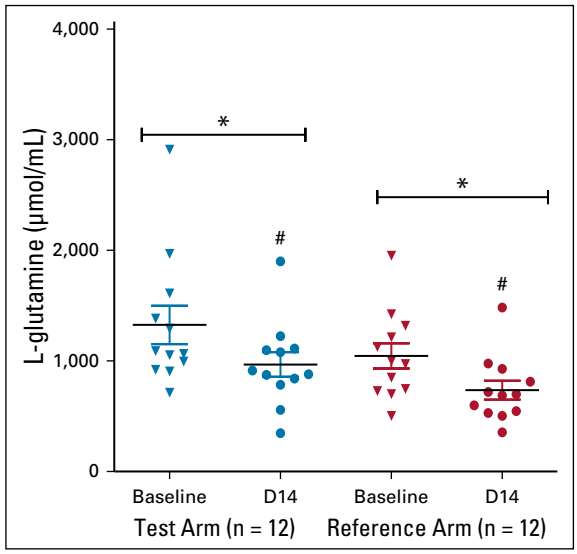

FIG 4. Change in plasma L-glutamine levels from baseline to day 14 (D14) in the test and reference arms. (*) P = not significant for change in glutamine levels from baseline to D14 after administration of either the test or reference product; (#) P = not significant for D14-glutamine levels in the test and reference arms.

aspartic acid levels on day 14. However, there was a nonsignificant increase in glutamate levels in both groups. Appel et al[27] have also observed an increase in the levels of aspartate and glutamate levels after pegaspargase administration in treatment of childhood ALL.

Both formulations were well tolerated; however, 83% of the patients in the study experienced toxicity of any grade. Causality assessment revealed that these toxicities were not related to pegaspargase; rather, they were attributable to concomitant drugs and to patients' disease status. An exception to this was a case of pancreatitis observed in a patient in the test arm. L-Asparaginase is known to cause hepatotoxicity[28]; however, none of the patients in either of the study arms developed any major hepatic dysfunction, although 2 patients in the test arm had grade 1 elevation of liver enzymes.

As a nonhuman protein, L-asparaginase is known to induce anti-asparaginase antibody formation in humans and sometimes results in silent inactivation and allergic

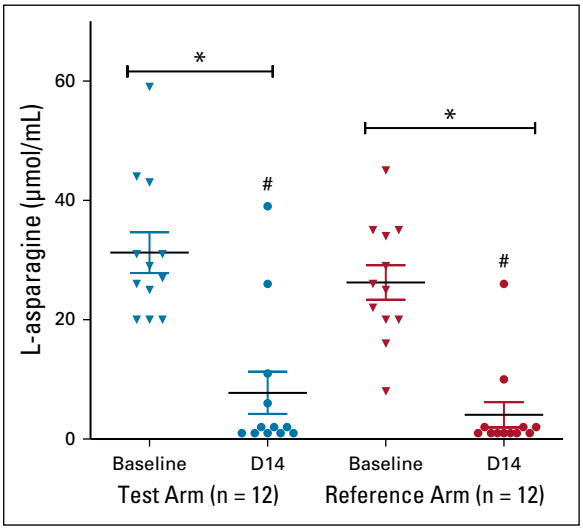

FIG 3. Change in plasma L-asparagine levels from baseline to day 14 (D14) in the test and reference arms. (*) $P < .05$ for change in asparagine levels from baseline to D14 after administration of either the test or reference product; (#) P = not significant for D14-asparagine levels in the test and reference arms.

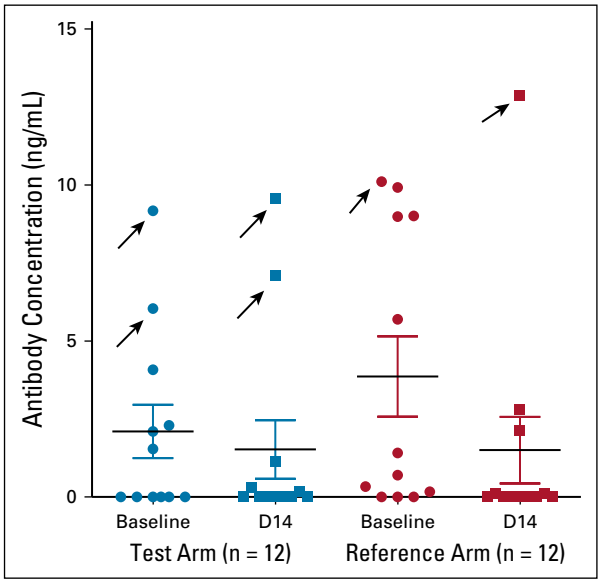

FIG 5. Anti–L-asparaginase antibody levels in the patients at baseline and day 14 (D14) in the test and reference arms. Arrows indicate patients who had consistently high levels of antibodies at baseline and D14.

TABLE 4. Percentage of Patients Having Grade 3/4 Nonhematological Adverse Events in the Test and Reference Arms

Adverse Event	Test Arm (n = 12)	Reference Arm (n = 12)
No. of patients experiencing any grade 3/4 adverse event	7 (58.33)	5 (41.67)
Sepsis	1 (8.33)	0
Febrile neutropenia	6 (50)	4 (33.33)
Hypotension	1 (8.33)	0
Sinusitis	1 (8.33)	0
Lung infection	0	1 (8.33)
Pancreatitis	1 (8.33)	0
Esophagitis	0	1 (8.33)
Mucositis	0	1 (8.33)

NOTE. Data presented as No. (%).

reactions.[2,8] Ko et al[29] found that approximately 30% of the patients treated with pegaspargase developed allergic reactions and anti-asparaginase antibodies. Circulating antibodies can lead to faster clearance of asparaginase, short duration of serum L-asparagine depletion, and subsequently poor response rates. Incidentally, none of the patients in the current study developed any hypersensitivity reactions. Because the study was conducted in patients with relapsed ALL, anti-asparaginase antibodies were expected in some patients, and we found that 58.6% of the patients were positive for anti-asparaginase antibodies at baseline. None of the patients in either study arm who had negative antibodies at baseline developed new antibodies by day 14. During the course of the treatment in both groups, the antibody titers had fallen to < 50% of their baseline levels in some patients and were depleted completely in a few others. However, in 3 patients (test arm [n = 2]; reference arm [n = 1]), antibody levels remained elevated at day 14. A study by Woo et al[30] also reported that anti-asparaginase antibody levels increase during post-reinduction.

In conclusion, generic pegaspargase (Hamsyl) was bioequivalent to the brand Oncaspar and was well tolerated in patients with relapsed ALL. This may be a safe and cost-effective alternative for patients with ALL, especially in LMICs.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

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No other potential conflicts of interest were reported.

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