Nepal J Biotechnol. 2021Dec.9 (2): 39-47 Review article DOI: ttps://www.doi.org/10.54796/njb.v9i2.41914

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: www.nepjol.info/index.php/njb ISSN (Print): 2091-1130

Efficacy and Toxicity of Different Forms of Asparaginases Against Acute Lymphoblastic Leukemia: A Review

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Received: 15th Jun 2021; Revised: 22nd Dec 2021; Accepted: 25th Dec 2021; Published online: 31st Dec 2021

Abstract

Acute lymphoblastic leukemia (ALL) is a form of blood cancer that affects white blood cells and is among the most common forms of leukemia with children and adolescents showing the highest number of cases. Most treatment protocols include chemotherapy using asparaginase. Asparaginase converts asparagine to aspartic acid and ammonia. Unlike normal, healthy cells, cancerous cells depend on asparagine for their growth. When these cells are deprived of asparagine by the action of the enzyme, the cancer cells selectively die. As of date, several forms of asparaginases are commercially available and are administered in ALL therapy. But due to limited study, it will be early and inaccurate to predict which forms of the enzymes are better. In this review, we aim to compare the efficacy and toxicity of four different asparaginases—native *Escherichia coli* asparaginase, PEG *Escherichia coli* asparaginase, *Erwinia chrysanthemi* asparaginase and a recombinant *Escherichia coli* asparaginase—used in ALL therapy in children and adolescents using available clinical trial data. PubMed and Clinical trial.org databases were used to select studies. Asparaginase activity, toxicity, anti-asparaginase antibody level and event-free, overall survival was compared for different asparaginases. Seventeen randomized and non-randomized controlled trials were included. Evidence was insufficient to ascertain which asparaginase is the best. PEG *Escherichia coli* asparaginase seems to be better with a high activity among the treated patients but there remains high toxicity for all available asparaginases. This study highlights a need to discover alternative sources of asparaginase from the organisms, which are evolutionarily distant from *Escherichia coli* and *Erwinia chrysanthemi* with high higher enzyme activity and reduced toxicity.

Keywords: Efficacy, Acute lymphoblastic Leukemia, Asparaginase, Clinical trials.

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Introduction

Acute lymphoblastic leukemia or ALL is among the most prevalent forms of leukemia and most commonly affects children. ALL is caused by unchecked and malignant proliferation of lymphoid progenitor cells in blood, bone marrow, and extramedullary sites [1]. ALL presents itself via three main pathological processes: first is the failure of bone marrow, second is the infiltration of other tissues by blasts via malignancy and third is the systemic effects arising from cytokines released by the cancerous cells. ALL often shows common signs and symptoms among children which include: anemia, thrombocytopenia, and pronounced hepatosplenomegaly or lymphadenopathy [1]. American Cancer Society estimates that 6590 cases were diagnosed in 2016 alone with 1400 death [2]. It is suspected that a combination of prenatal mutations and environmental factors cause ALL.

L-asparaginase is an enzyme that catalyzes the formation aspartic acid and ammonia from asparagine. It is commonly produced in bacteria, but not in humans. Organisms produce it in the course of their normal life cycle and it can be extracted and purified for industrial and medical purposes. L-asparaginase was introduced in the early 70s as part of the treatment protocols for ALL in children. Now, L-asparaginase has become a routine part of treatment protocols of ALL [3]. Asparaginase works via depletion of asparagine in the blood. L-asparagine is an important amino acid used by cells in protein synthesis. Most normal cells produce L-asparagine for their growth using L-asparagine synthetase enzyme, synthesizing L-asparagine from aspartic acid and glutamic acid. Neoplastic cells like ALL cells are incapable of producing their own L-asparagine because they lack L-asparaginase synthetase enzyme. Thus, they are dependent on the extracellular supply of asparagine for their existence and reproduction. Exogenous source can be the serum where asparagine from diet and normal cells are pooled. Consequently, L-asparaginase is used as a therapeutic agent against ALL. L-asparaginase catalyzes the degradation of asparagine into ammonia and aspartate and depletes the asparagine in the blood serum, which leads to starvation of cancer cells causing cell death [4].

L-asparaginase is commercially extracted mostly from *Erwinia chrysanthemi* and *Escherichia coli*. The enzyme can be used in its purified native state or can be

conjugated to increase its half-life. PEG *Escherichia coli* asparaginase is a variant made by conjugating the *Escherichia coli* L-asparaginase with polyethylene glycol. There are many commercially available examples of PEG *Escherichia coli* asparaginase as well. Usually, PEG-asparaginase can be used in lower doses compared to native state asparaginases due to its greater half-life [5]. Some people can have allergic reactions to *Escherichia coli* asparaginase. *Erwinia chrysanthemi* asparaginase is used as an alternative for these cases [3].

Therapeutic results of combining L asparaginase with chemotherapy protocols have been generally very successful. Many variations have been made to improve the results of these therapies, usually centered around reducing enzyme related side effects that are common in these therapies[6]. Though all three forms of L asparaginase have been extensively studied for their effectiveness and safety, unintended enzyme-related side-effects like hypersensitivity and allergic reactions abound [7]. Till date, there has been limited study to address the efficacy and toxicity of different forms of asparaginases available for therapy because of which individuals who are under therapy are at high risk. This creates an urgent need to address and minimize the risk by providing necessary and relevant information on safety and fill in the research gap. In this study, we have attempted to compile and examine a list of studies to understand efficacy, safety and toxicity of PEG *Escherichia coli* asparaginase, *Erwinia chrysanthemi* asparaginase, and native *Escherichia coli* asparaginase to determine if there is a need for an alternative source of asparaginase to reduce these unwanted side effects.

Asparagine Concentration:

Ogawa et al. reported that the average plasma asparagine concentration of patients treated with *E. chrysanthemi* asparaginase was 0.218 μM [8]. Concentration of < 0.5 μM is considered as complete depletion of asparagine from the blood [9]. Van der Sluis et al reported that complete depletion of asparagine was seen in 97.8% of patients treated with recombinant asparaginase and 97.9% of patients treated with native *E. coli* asparaginase [10]. Likewise, Pieters et al. claimed that the mean asparagine concentration dropped to 0.5 µM under both recombinant *E. coli* asparaginase and native *E. coli* asparaginase in 99% of patients [11]. In another study by van der Sluis et al. the mean asparagine concentration dropped to <0.5 μM in all patients at all time points measured with recombinant *E. coli* asparaginase[12].

In a comparison done between intermittent and continuous routes of PEG *E. coli* asparaginase among 625 children of Europe, it was found that 95% of patients were asparagine depleted during treatment [13]. In 2011, a study conducted to demonstrate PEG *E. coli* asparaginase as a viable alternative in patients that have shown allergic reactions to treatments using native *E. coli* asparaginase, it was found that asparagine level depleted to 40% and 20% at day 7 and day 14 respectively for hypersensitive patients using PEG asparaginase. Similarly for non-hypersensitive patients, it was depleted to 50% at day 14 but while using native *E. coli* asparaginase it was depleted to only 86% on day 14 [14]. 26% of patients receiving *E. chrysanthemi* asparaginase showed asparagine depletion during re-induction. With *E. coli* asparaginase receiving patients, asparagine depletion was seen in 60% to 90% during the re-induction phase. Serum asparagine levels recovered after 4 days for patients administered with *E. chrysanthemi* asparaginase compared to 11 days for *E. coli* asparaginase [15] (**Table 1**).

Table 1. Average asparagine Concentration in treated patients from individual trials

Clinical Trial	Type of Asparaginase	Average Concentration(μM)
[8]	*Erwinia chrysanthemi*	0.218
[16]	Native *Escherichia coli*	0.13
[10]	Native *Escherichia coli* (asparaginase medac)	<0.5
[10]	Recombinant *Escherichia coli*	<0.5
[17]	Native *Escherichia coli* (asparaginase medac)	<0.5
[17]	Recombinant *Escherichia coli*	<0.5
[12]	Recombinant *Escherichia coli*	<0.5
[13]	PEG *Escherichia coli*	<0.5
[14]	Native *Escherichia coli*	<0.5

Asparaginase Activity:

Measurement of asparaginase concentration during the therapy has lots of technical limitations, which is why asparaginase-enzyme activity is generally used to monitor asparaginase. As per U.S FDA, effective plasma level of asparaginase was defined as ≥ 0.1 IU/mL and used for determination of efficacy in the approval process for asparaginase [18]. Ogawa et al. reported that the average activity of *E. chrysanthemi* asparaginase in treated patients throughout the study was 0.36 IU/mL, which was much higher than the therapeutic level of asparaginase [8]. All the treated patients in their study achieved a therapeutic level of asparaginase. Likewise, Vyas et al. noted in patients treated with native *E. coli*

asparaginase and PEG *E. coli* asparaginase, the activity of the enzymes were 0.13 IU/mL for native *E. coli* asparaginase and 0.30 IU/mL for PEG *E. coli* asparaginase with 86% in the native *E. coli* group and 94% generic PEG *E. coli* group achieving a therapeutic level of asparaginase [16]. In case of recombinant *Escherichia coli* asparaginase, Van der Sluis et al. reported that the average asparaginase activity was 0.17 IU/mL with 62.2 % of patients achieving the therapeutic level of asparaginase and in native *E. coli* asparaginase average asparaginase activity was 0.16 IU/mL with 65.9% patients achieving the therapeutic level of asparaginase [10]. Similarly, Place et al. claimed that PEG *E. coli* asparaginase activity was around 0.7 IU/mL is treated patients and native *E. coli* asparaginase activity was around 0.1-0.2 IU/mL[19].

In a phase II study conducted by Dinndorf et al. for the FDA, the asparaginase activity and the depletion of asparagine were measured in days after the first dose. They found that between the 2nd and 7th day after the first dose both native *E. coli* asparaginase and the PEG *Escherichia coli* asparaginase had activities above 0.03 IU/mL in 50 patients. This number decreased to below 10 patients for native *E. coli* asparaginase group while it reached 20 for the PEG group in the remission induction phases [20].

In a study, the therapeutic PEG *E. coli* asparaginase activity was observed to be 0.234 IU/mL among 86% of surviving patients [17]. In another study by Pieters et al. median asparaginase activity for native *E. coli* asparaginase was found to be 0.19 IU/mL while recombinant asparaginase showed an activity of 0.14 IU/mL (Pieters et al. 2008). Moreover, Van der Sluis et al. reported the serum asparaginase activities of recombinant *E. coli* asparaginase to be >0.10 IU/mL in 74% patients and was 0.13 IU/mL of all measured samples respectively [12]. In Rau et al. none of the study population completed the trail and only one patient had tolerated the PEG *E. chrysanthemi* asparaginase with activity >0.1 IU/mL [21]. Significantly shorter serum half-life of 0.65 days was observed for *E. chrysanthemi* asparaginase enzyme compared to 1.24 days for *E coli* asparaginase in a study by Duval et al. [15] (**Table 2**).

As stated earlier, asparaginase activity in-vivo was standardized to be less than 0.1 IU/mL during the therapy, but it happens to be between 0.13-0.70 IU/mL (Table 3). This showcases that most of the individuals, who were under therapy achieved the threshold.

Toxicity:

In a therapy involving asparaginase, toxicity is directly related to the dose administered. This is why controlled administration of quantity is a must. A trial reported that *E. chrysanthemi* asparaginase hypersensitivity reaction (urticaria) of grade 1-2 were seen in 2(8%) of patients, pancreatitis of grade 1-3 in 3(12%) of the patients, and hyperglycemia of grade 1-2 in 5 (20%) of the patients [8]. Similarly, hypersensitivity reaction of grade 3-4 was seen in 7(12%) patients in native *E. coli* asparaginase treated group, 3 (6%) in PEG *E. coli* asparaginase treated group, hyperglycemia of grade 3-4 was seen in 2(4%) patients in native *E. coli* asparaginase treated group and in 1(2%) patient in PEG *E. coli* asparaginase group [16]. Later, Van der Sluis et al reported that hypersensitivity reaction was seen in 2(2.1%) of the patients in recombinant *E. coli* asparaginase group and 5(5%) in native *E. coli* asparaginase group, pancreatitis of grade ≥ 2 was seen in 1 (1%) of patient in native *E. coli* asparaginase group [16]. Also the hypersensitivity reaction of grade 1-4 was seen in 28(12%) of patients receiving PEG *E. coli* asparaginase and in 21(9%) in native *E. coli* asparaginase receiving group. Pancreatitis of grade ≥ 2 was seen in 27 (12%) of the patients receiving PEG asparaginase and 22(10%) receiving native *E. coli* asparaginase group [19].

In 1999, in a study by Liang et al. 10,000 IU/m2 dose *Escherichia coli* asparaginase was used during the remission induction therapy on 93 children with

Table 2. Average asparaginase activity from individual trials in treated patients

Clinical Trial	Type of Asparaginase	Average Activity (IU/mL)
[8]	*Erwinia chrysanthemi*	0.36
[16]	Native *Escherichia coli*	0.13
[16]	PEG *Escherichia coli*	0.30
[10]	Native *Escherichia coli* (asparaginase medac)	0.16
[10]	Recombinant *Escherichia coli*	0.17
[19]	Native *Escherichia coli*	0.1-<0.2
[19]	PEG *Escherichia coli*	0.70
[17]	Native *Escherichia coli* (asparaginase medac)	0.19
[17]	Recombinant *Escherichia coli*	0.14
[12]	Recombinant *Escherichia coli*	0.13

Table 3. Average range of asparaginase activity in treated patients from all the studied trials.

Type of asparaginase	Average Activity Range (IU/mL) from all trials
Native *Escherichia coli*	0.13-0.19
PEG *Escherichia coli*	0.23-0.70
Erwinia chrysanthemi	0.13-0.58
Recombinant *Escherichia coli*	0.13-0.17

standard-risk or SR (Spontaneous Remission) ALL. They found that 26.8% or 25 of the participants showed signs of toxicity. Of them, 15 or 16% showed signs of sepsis, 2 or 2% had pneumonia, 6 or 6% showed signs of hyperglycemia, and 6 or 6% had hemorrhage. During remission induction, 19 of 93 or 20.4% of patients developed a severe infection. Death during induction occurred in 6 patients [22]. A phase II clinical trial was conducted by Dinndorf et al. for the PEG *Escherichia coli* - asparaginase Oncaspa® by Enzon Pharmaceuticals in 118 children aged 1 to 9 years. It was a comparative study between a native *E. coli* asparaginase and the PEG *E. coli* asparaginase. They concluded, 14 of the 58 patients in the PEG asparaginase group compared to 18 of the 59 patients in the native *Escherichia coli* group suffered from toxic effects. Hyperglycemia was much more common in the PEG *Escherichia coli* asparaginase group with 5% or 3 patients suffering from it and abnormal liver conditions were much more common in the native *Escherichia coli* asparaginase group, 10 patients with abnormal liver tests compared to 6 in the PEG *Escherichia coli* asparaginase group [20].

Another study conducted on 144 patients aged below 22 years to study the effect of weekly vs. bi-weekly dosing regimens with PEG *E. coli* asparaginase and native *E. coli* asparaginase in patients with first relapses of ALL in reinduction therapy, found that out of the 143 patients whose data was evaluated 72 or 50% of them suffered from severe infections. 29 of them had hypoalbuminemia, 32 of them had low fibrinogen and 9 of them showed weight loss.

There were also specific toxicities seen in the group given PEG *E. coli* asparaginase. Only 6 of the 144 tested showed PEG-asparaginase hypersensitivity, 4 of whom only showed grade I allergic reaction. The other 2 had grade III hypersensitivity and were given alternative *E. chrysanthemi* asparaginase instead [23].

Additionally, in the *E. coli* asparaginase 5000 arm 9 (2.7%) deaths were observed, whereas in *E. coli* asparaginase 10000 arm 23 (6.5%) deaths were observed. Pneumonia was seen in about 50% of patients and hypersensitivity reaction was reported in 4.5% (n=31) patients in *E. coli* asparaginase 10000 arm. In *E. coli* 5000 arm 1.8% (n=13) patients showed hypersensitive reaction [24]. Later, 15 out of 16 deaths were for patients over 40 years. Sepsis together with hepatotoxicity occurred in 50% of the dead patients. Among surviving people treated with PEG *E. coli* asparaginase pancreatitis and hypoalbuminaemia of grade 3+ were recorded on 2 patients (3%) [17]. Pieters et al. reported no death during the trail but recorded deep venous thrombosis and severe hyperglycemia in two separate patients given with recombinant asparaginase and similarly, deep venous thrombosis and severe neutropenia in two different patients treated with native *E. coli* asparaginase [11]. A study conducted by Van der Sluis et al. found that 12 patients under treatment showed hemorrhage, nose bleeding, thrombosis of the superior vena cava and increased alanine aminotransferase CTC grade III [12]. Rau et al. reported complications of chest tightness and facial erythema with mild swelling and anaphylaxis in three patients [21].

Albertsen et al. in 2019, demonstrated that 60 (9.6%) patients experienced toxicity during PEG *E. coli* asparaginase treatment and 23 (3.7%) after the last dose. Among those showing symptoms, hypersensitivity was seen in 13 (2%), osteonecrosis in 29 (4.6%), pancreatitis was seen in 24 (3.84%) and thromboembolisms in 17 (2.72%). In a 3-year period of observation, incidence of any form of toxicity associated with first asparaginase treatment after randomization was found to be much higher in children of age 10 years or older compared to children younger than 10 years, but did not differ between boys and girls or between patients at intermediate risk or standard risk [13]. Additionally in another study comparing native *E. coli* asparaginase with *E. chrysanthemi* asparaginase, *E. coli* asparaginase arm had more instances of coagulation abnormalities (30.2%) compared to *E. chrysanthemi* asparaginase arm (11.8%) [15].

Another trial conducted in Spain by Ribera et al concluded that during induction therapy, percentage of patients with detectable infection, hypersensitivity, thrombosis, hepatic toxicity, pancreatitis and coagulopathy (all of them within grade 3-4) for native *E. coli* asparaginase was 56%, 1%, 6%, 21%, 1%, 11% respectively while for PEG *E. coli* asparaginase was 45, 0, 9, 38, 3, 18 respectively. Similarly during consolidation therapy, percentage of patients with detectable infection, hypersensitivity, thrombosis, hepatic toxicity, pancreatitis and coagulopathy (all within grade 3-4) for native *E. coli* asparaginase was 16%, 1%, 0.4%, 3%, 0%, 0.4% respectively while for PEG *E. coli* asparaginase was 13%, 1%, 0%, 11%, 0%, 5% respectively [25].

Toxicities caused by different forms are asparaginases are relatively similar in every patient group (**Table 4**).

Table 4. Average range of occurring toxicity in treated patients from all the studies trials.

Asparaginase Types / Toxicity	*Escherichia coli* Asparaginase (%)	PEG- *Escherichia coli* Asparaginase (%)	*Erwinia chrysanthemi* Asparaginase (%)	Recombinant *Escherichia coli* Asparaginase (%)
Hypersensitivity	1.8 – 12	2– 12	1.3 – 12	2.1
Pancreatitis	1 – 10	3-12	NA	NA
Hyperglycemia	4 – 6	2	5 – 20	NA

The range of toxicities are of 1-4 grade from all the trials.

Event-Free Survival (EFS):

Following initial treatment for cancer, patients generally remain free of any complications or symptoms that were intended to delay or prevent their treatment. This is known as an event-free survival. Vyas et al. reported that 2-years event-free survival of patients treated with PEG *E. coli* was 84% and treated with native *E. coli* asparaginase was 80.7% [16]. Place et al evaluated 5-years of event-free survival of patients to be 90% in PEG *E. coli* asparaginase treated group and 89% native *E. coli* asparaginase treated group[19].

Pession et al. study found that 5 year and 10 year event-free survival (EFS) of native *E. coli* asparaginase group was 84.6% and 82.5% for the 494 patients enrolled in the trial. 58 cases failed to achieve event-free status with 1 case of second malignancy and 22 relapses. The study by Liang et al. was a comparison between the use of native *E. coli* asparaginase and epidoxorubicin in the treatment of SR ALL in the remission induction therapy. They also saw 5 relapses in their asparaginase arm of the study group. They estimated an EFS at 3 years to be 72%[22].

The FDA phase II trial study by Dinndorf et al. did not attempt to find long-term EFS. They estimated an 80% EFS for both their Erwinia asparaginase group and PEG asparaginase group. Karachunskiy et al. conducted a study on event-free survival rates and found that at 10 years the probabilities of event-free survival rates for *Escherichia coli* 5000 arm (79 ± 2%) were not significantly different from *Escherichia coli* 10000 arm (75 ± 2%) [24]. While comparing native *E. coli* asparaginase with *E. chrysanthemi* asparaginase, event-free survival predicted was 6 years and percentage patient survival was 73.4% versus 59.8% [15] (**Table 5**).

By observing years of event free survival ranges, it can be discerned that PEG *E. coli* asparaginase gives low degree of disease reoccurrence (**Table 6**).

Follow up period for event free survival patients were reported for 10 years for native *E. coli* (10 years), 6 years for PEG *E. coli* and 6 years for *E. chrysanthemi* asparaginases.

Overall Survival:

A clinical trial reported 2-year event-free survival of patients at 93% treated with PEG *E. coli* asparaginase and 84% treated with native *E. coli* asparaginase [16] and another trial evaluated 5-years of overall survival of patients. The statistics was 96% treated with PEG-asparaginase and 94% treated with *Escherichia coli* asparaginase [19]. Likewise, Karachunskiy et al. reported that patients of *E. coli* asparaginase 5000 arm (86 ± 2%) had slightly superior probability of overall survival at 10 years compared to *E. coli* asparaginase 10000 arm (82 ± 2%) [24]. Overall survival estimated at 5 and 10 years was 94.4% and 93.7% in the group with asparaginase versus 89.8% and 88.6% in the group without asparaginase , respectively [26] (**Table 7**).

Moreover, study conducted in 2002 demonstrated that 6 year survival rate using native *E. coli* asparaginase was greater than using *E. chrysanthemi* asparaginase (83.9% vs 75.1%) [15].

Table 5: Time period of Event-Free Survival in treated patients from individual trials.

Clinical trial	Type of Asparaginase	Event free survival year	% of patients survived	Follow up period (Years)
[16]	Native *Escherichia coli*	2	80.7	2
	PEG *Escherichia coli*	2	84	
[19]	Native *Escherichia coli*	5	89	6
	PEG *Escherichia coli*	5	90	
[26]	Native *Escherichia coli*	5	84.6	10
	Native *Escherichia coli*	10	82.5	
[24]	Native *Escherichia coli*	10	73-81	10
[15]	Native *Escherichia coli*	6	73.4	6
	Erwinia chrysanthemi	6	60	
[22]	Native *Escherichia coli*	3	72	3

Table 6: Average year range of Event-Free Survival in treated patients from all the studied trials.

Type of asparaginase	Average year range of Event-Free Survival Year (% range)
Native *Escherichia coli*	5(84%-89%)-10 (75%-82%)
PEG *Escherichia coli*	5(90%)
Erwinia chrysanthemi	6 (59%)

Table 7. Time period of Overall Survival in treated patients from individual trials.

Clinical trial	Type of Asparaginase	Overall survival 1 year	% of patients survived	Follow up period (Years)
[16]	Native *Escherichia coli*	2	84	2
	PEG *Escherichia coli*	2	93	
[19]	Native *Escherichia coli*	5	94	6
	PEG *Escherichia coli*	5	96	
[26]	Native *Escherichia coli*	5	94.4	10
	Native *Escherichia coli*	10	93.7	
[24]	Native *Escherichia coli*	10	80-88	10
[15]	Native *Escherichia coli*	6	84	6
	Erwinia chrysanthemi	6	75	

In terms of overall survival PEG asparaginase from *Escherichia coli* showed better result than other forms of asparaginases (**Table 8**)

Table 8. Average year range of Overall Survival in treated patients from all the studied trials.

Type of asparaginase	Average year range of Overall survival Year (% range)
Native *Escherichia coli*	5 (94%) - 10 (93%)
PEG *Escherichia coli*	5 (96%)

Follow up period of overall survival of patients were reported for 10 years for native *E. coli* asparaginase, 6 years for PEG *E. coli* asparaginase and 6 years for *E. chrysanthemi* asparaginase.

Anti-Asparaginase Antibody (AAA)

The most common adverse reactions of asparaginase in children are produced by anti-asparaginase antibodies. These adverse reactions can manifest as mild or severe allergic reactions. It was reported that 10% (9 in native *E. coli* asparaginase and 10 in recombinant *Escherichia coli* asparaginase group) patients were detected positive for AAA [10]. The Dinnodorf et al. FDA study found that 16 out of 57 or 28% of their patients treated with native *E. coli* asparaginase had anti-aparaginase antibodies at any given time in of the treatment. Three subjects were known to have pre-existing anti-aparaginase antibodies. In the patients treated with the PEG *E. coli* asparaginase 11% of the 55 or 6 of them had asparaginase antibodies. Rau et al. 2018 reported very low anti-PEG IgM among three patients. Anti-PEG IgG was observed in three patients except one after 5.5-years of exposure to PEG *E. coli* asparaginase [21] (**Table 9**).

Table 9. individual patients positive with Anti-Asparaginase Antibody (AAA) from trials.

Clinical Trial	Type of Asparaginase	% of patients positive for Anti-Asparaginase Antibody (AAA)
[10]	Recombinant *Escherichia coli* Native *Escherichia coli* (Asparaginase medac)	10
[20]	Native *Escherichia coli*	28
	PEG *Escherichia coli*	11

PEG asparaginase from *Escherichia coli* has shown promising advantages over other forms of asparaginases. Anti-asparaginase antibodies are found in lower numbers in PEG asparaginase . (**Table 10**).

Table 10. Average % range of patients positive for Anti-Asparaginase Antibody (AAA) from all the studies trials.

Type of asparaginase	Average % range of patients positive for Anti-Asparaginase Antibody (AAA)
Native *Escherichia coli*	10%-28%
PEG *Escherichia coli*	11%

Discussion and Conclusion

Data was obtained from various clinical trials (Table 11) on asparaginase activity, concentration, and toxicity of the three major types of asparaginases used for ALL therapies: PEG-asparaginase, *Erwinia chrysanthemi* asparaginase and native *Escherichia coli* asparaginase. PEG *E. coli* asparaginase activity was seen to be between 0.3-0.7 IU/mL, which is the highest in comparison to others (**Table 3**). In terms of toxicity, all three forms of asparaginases showed similar results (**Table 4**). Toxicities like hyperglycemia and pancreatitis were seen in a significant number of cases leading to a decrease in the effectiveness of the enzyme in the treatment of the patients. In several studies, we can see that incidence of toxicity increases with the dose of the enzyme. Another minor conclusion that we can derive from clinical trials by Place et al and Vyas et al is that overall survival was slightly higher for PEG *E. coli* asparaginase than native *E. coli* asparaginase (**Table 8**). This difference is very slight and the results stay the same for event free survival making conclusions about the higher efficacy of one type of asparaginase over the other difficult (**Table 6**).

There were number of other studies that did not directly evaluate the safety and efficacy of asparaginases in clinical trials for children. Since these studies were also included in this review, we will discuss the findings of these studies. In the study by Rau et al. pegcrisantaspase (Erwinia *chrysanthemi* pegylated asparaginase) treatment also reported hypersensitivity reaction to the patients

with previously showing hypersensitivity reaction to PEG asparaginase treatment.

Table 11. General data of selected studies.

Author, year	Country	Type of Enzyme (Asparaginase)	Route of administration	Period of treatment of Enzyme	Total Number of Participant	Study group(age)
Ogawa et al. 2017	Japan	*Erwinia chrysanthemi*	Intramuscular	2weeks	24	2-16 years
Vyas et al. 2018	India	Native *Escherichia coli*) generic PEG *Escherichia coli)*	Intramuscular Intravenous	10 weeks	106	Less than 18 years
Van der Sluis al, 2018	Netherlands	Recombinant *Escherichia coli* Native *Escherichia coli*	Intravenous	5 weeks	199	Children
Place et al. 2015	USA and Canada	PEG *Escherichia coli*) *Escherichia coli*	Intravenous Intramuscular	30 weeks	463	1-18 years
Pession et al. 2005	Italy	90% of the patients received *Erwinia chrysanthemi* 10% *Escherichia coli*	Intramuscular	24 months	490	1-15 years
Liang et al. 1999	Taiwan	*Escherichia coli*	Intramuscular	110 weeks	201	1-15 years
Dinndorf et al. 2007	USA	*Escherichia coli*[Elspar® from Merck] PEG *Escherichia coli*Oncaspa® By Enzon Pharmaceuticals, Inc	Both intramuscular	12 Weeks	118	1-9 years
Abshire et al. 2000	USA	PEG *Escherichia coli* *Erwinia chrysanthemi*	Intramuscular	4 weeks	144	Below 22 years of age
Karachunskiy et al	Moscow–Berlin	Native *Escherichia coli*	Intramuscular	200 days	774	1-19 years
Patel et al. 2017	UK	PEG *Escherichia coli*	-	8 weeks	91	25-65 years
Pieters et al.	Netherlands	Recombinant *Escherichia coli* asparaginase Asparaginase medac	Intravenous	39 days	32	1-14 years
van der Sluis et al. 2013	Netherlands and Germany	Recombinant *Escherichia coli* -asparaginase	-	39 days	12	Below 1 years
Rau et al. 2018	USA	PEG *Erwinia chrysanthemi*	Intravenous	29 days	4	1-20 years
Albertsen et al. 2019	Denmark, Finland, Iceland, Norway, or Sweden	PEG- *Escherichia coli*	Intramuscular	30-33 weeks	625	Children
Duval et al. 2002	Belgium, France and Portugal	*Escherichia coli* *Erwinia chrysanthemi*	Intravenously	6 weeks	700	Less than 18 years
Kurtzberg et al. 2011	USA, Canada	PEG *Escherichia coli* asparaginase Native *Escherichia coli*	Intramuscularly	4 weeks	76	Less than 21 years
Ribera et al. 2017	Spain	Native *Escherichia coli*	Intravenously	4 weeks	126	18-60 years

It is possible that PEG (poly ethylene glycol) can be immunogenic and anti-PEG IgG antibodies are formed during PEG-asparaginase treatment. The remaining immunological memory may mediate hypersensitivity reaction during pegcrisantaspase treatment. One of the patients involved in this study had not been exposed to pegaspargase for 5.5 years. He did not experience a pegcrisantaspase hypersensitivity reaction. A lack of a durable immunologic memory from anti-PEG-mediated immune reactions may be the case for this patient. It is suggested that patients who have been recently exposed to PEG in the formulation of other medicine in any disease should not use any PEG asparaginase in any form. Instead, native *Escherichia coli* or *Erwinia chrysanthemi* asparaginase should be used.

In another study by Patel et al. it was shown that asparaginase toxicity can be substantial in older patients, making it difficult to deliver safely to those aged above 40 years [17]. Similarly, when Ribera et al used native or PEG asparaginase in adult patients there no significant difference observed in complete remission, diseases free survival, and overall survival with no influence in patient response and outcome [25]. Based on numerous previous studies teenagers and younger adults typically have better outcome from induction and consolidation treatment compared to adults (aged above 40 years). Careful timing of administration and avoidance of overlapping toxicities are recommended for the older patients.

In the Liang et al. study authors state that the increased mortality was due to immunosuppression via depletion of blood asparagine by the enzyme. They had increased the doses of the Leunase brand asparaginase to match that of the dose preparation by Nesbit et al. of *Escherichia coli* preparation called Crasnitin. Authors attribute the severe infection and unexpected mortality to this dose change [22]. The product sold as Medac, is also known to cause excessive toxicity when given at a high dose, leading to a 50% reduction in the dosage [27]. Karachunskiy et al. have reported that patients treated with *E. coli* asparaginase of dose 10000 U/m^2 have reported the severe hypersensitivity reaction more frequently than patients provided with 5000 U/m^2 of dose. Also, death in complete remission occurred significantly more in 10000 U/m^2 provided patients [24]. An advantage of higher doses was not found in the group.

Thus, it will be advantageous to discover and use a higher activity asparaginase, thereby allowing use of lower dose of enzyme to reduce the incidences of toxicity. Enzymes with low Km value will have higher activity against L-asparagine. Besides the L-asparagine activity of L-asparaginase, there is the secondary activity of mentioned asparaginase enzymes against L-glutamine, that has been linked to the different toxic side effects[28,29]. Also, the role of L-glutamine activity has not been seen in anti-cancer activity of the enzyme[30]. One method of finding new asparaginase is to extract it from an organism other than *Escherichia coli* or *Erwinia chrysanthemi*. We can expect an organism that is evolutionarily distant from *Escherichia coli* or *Erwinia chrysanthemi* to have different enzyme activity. Thus, we can expect to find better alternatives to commercially available asparaginases with higher activity than *Escherichia coli* and *Erwinia chrysanthemi* [31, 32].

According to this study, PEG asparaginase provides better enzyme concentration than *E. coli* or *Erwinia chrysanthemi* asparaginase in various clinical trials. Similarly, two studies show that PEG asparaginase has higher 2-year overall survival than native *E. coli* asparaginase. The difference is very minor to conclusively say PEG asparaginase is superior. Using *Erwinia chrysanthemi* asparaginase when *E. coli* and PEG asparaginase fail, as is currently done, is recommended from this study as well. Furthermore, alternative source of asparaginase from the organisms, which are evolutionarily distant from *Escherichia coli* and *Erwinia chrysanthemi* and with a lower Km value i.e., higher enzyme activity toward L-asparagine, and low activity towards the L-glutamine need to be discovered. Such novel asparaginases can be used in lower dose thereby by reducing toxicity.

Acknowledgement

The authors acknowledge the Department of Biotechnology, Kathmandu University, Dhulikhel, Nepal for providing all support during the study period.

Competing Interests

The authors declare no competing interests.

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