The Effect of L-Asparaginase Therapy on the Activity of Factor II (Thrombin) in Children with Acute Lymphoblastic Leukemia

Aseel Nashat Alkhalidi* FICMS

ABSTRACT

Background: L-asparaginase is one of the chemotherapeutic drugs used to treat acute lymphoblastic leukemia (ALL) especially in children. It has an adverse effect that causes bleeding and thrombosis. It has a profound effect on hepatic synthesis of coagulation and fibrinolytic factors such as fibrinogen, factors II, VII, IX, X, XI and antithrombin.

Objective: To study the activity of FII (thrombin) before and after using L-asparaginase therapy in childhood ALL during the induction phase.

Methods: In a prospective interventional study in 2011, 30 newly diagnosed children with ALL were tested for factor II activity before and after taking this medication during the induction phase. Eighteen of them were collected from Medical City, Children Welfare Teaching Hospital and 12 of them were collected from Central Child Teaching Hospital at Al-Eskan. The results were compared to 30 healthy children age and gender matched as a control group.

Results: There was no significant difference between the activity of factor II before and after administration of this drug in this study with mean factor II activity before and after treatment 96.5±1.86 and 97.83±1.71%, respectively, (P=0.566).

Conclusion: Factor II activity did not show significant changes in childhood ALL patients after taking L-asparaginase in this study.

Keywords: Factor II, Thrombin, L-Asparaginase, Childhood acute lymphoblastic leukemia.

Iraqi Medical Journal Vol. 66, No. 1, Jan-July 2020; p.57-60.

Generally, leukemia is the most common childhood cancer and acute lymphoblastic leukemia (ALL) is the most common subtype, accounting for 23% of all cancers and 76% of all leukemias in patients younger than 15 year [(1)].

Treatment is generally divided into three phases, namely, induction, intensification (consolidation), and maintenance[(2)]. During the induction phase, therapy is usually given for four weeks and consists of vincristine weekly, a corticosteroid such as dexamethasone or prednisone, and l-asparaginase[(3)].

There are three different preparations of L-asparaginase with significantly different half-lives are available:

1- Native E. coli (1.2 days).

2- Erwinia (0.65 days).

3- PEG (Polyethylene glycated) - L - asparaginase (5.7 days)[(4)].

L-asparaginase has an adverse coagulopathic effects that cause both bleeding and thrombosis. This drug has a profound effect on hepatic synthesis of coagulation and fibrinolytic factors[(5)]. This results in decreased levels of coagulation factors II, VII, IX, X and fibrinogen[(6)], as well as histidine-rich glycoprotein, α-2 macroglobulin, and α-2 antiplasmin, producing an increased bleeding risk. The incidence of bleeding complications is low. One reason for this low frequency is the concurrent impaired synthesis of naturally occurring anticoagulant proteins, including antithrombin, protein C and protein S, and plasminogen. The incidence of thrombo-embolic complications is around 2% to 10% in adults. However, in children treated for

*Dept. of Laboratories, Hematopathology, Children Welfare Teaching Hospital, Medical City, Baghdad, Iraq
E-mail:dr.aseel.hemato@gmail.com

ALL with L-asparaginase, a much higher rate of 36.7% was found. Most thrombosis was asymptomatic[5].

Prothrombin (coagulation factor II) is a vitamin-K dependent co-factor which is activated by factor Xa to form thrombin. α-thrombin (F IIa) is the most potent activator of circulating platelets. Thrombin cleaves fibrinogen to generate the fibrin clot, converts factor XIII to factor XIIIa to cross-link and stabilize the clot, the proco-factors V and VIII are activated by α-thrombin as are factor XI and, potentially, factor VII[7]. Thrombin bound to thrombomodulin activates protein C, an inhibitor of the coagulation cascade. Activated protein C inactivates factors Va and VIIIa. Thrombin is also inactivated by antithrombin, a serine protease inhibitor[8]. Markedly decreased levels of α-thrombin is often characterized by bleeding diatheses Conversely, elevated levels of α-thrombin promote the risk of thrombosis[7].

The aim of this study is to study the activity of FII (thrombin) before and after using L-asparaginase therapy in childhood ALL during the induction phase.

Methods

During a period of 5 months (from May 2011 to October 2011), an interventional prospective study was done where 30 newly diagnosed pediatric patients with ALL were included. Eighteen of them were collected from Medical City, Children Welfare Teaching Hospital and 12 of them were collected from Central Child Teaching Hospital at Al-Eskan. Results were compared to a group of healthy 30 children age and sex matched. For all those 30 patients, blood sample was taken before starting treatment with L-asparaginase (i.e. before induction) to perform Factor II activity assay. The same test was re-done for the same patients after they had completed 9 cycles of 6000 IU/m^2 L–asparaginase which was given three times per week according to treatment protocol UKALL 2003 (The treatment protocol that was used for the patients included in the study). The formula used is of the native type E. coli derived.

Plasma FII was assayed using commercially kit (Deficient II substrate plasma for factor II assay REF 00745 /Diagnostica Stago). A calibration curve for each test–run constructed, standard required for the calibration line were prepared using Owren koller buffer and plastic test tubes to prepare standard according to the following dilution scheme: 1:10 dilution represent the assay value (i.e. 100%). 1:20 dilution represent half the assay value (i.e. 50%). 1:40 dilution represent one-fourth the assay value (i.e. 25%).

Patients' plasma: Using Owren-Koller buffer and plastic tubes to prepare 1:10 dilution, 1:20 dilution and 1:40 dilution[9].

Analysis of data was carried out using the available statistical package of SPSS-18 (Statistical Packages for Social Sciences- version 18 "PASW" Statistics). The significance of difference of different means (quantitative data) was tested using independent student-t-test. Statistical significance was considered whenever the P value was less than 0.05.

Results

The age of the patients was from 2-11 years with mean age 5.32±2.80 years. The largest group (11 out of 30 patients, 36.7%) was from 2-3 years. Nineteen patients (63.3%) were males and 11 patients (36.7%) were females.

The age of the control group were from 2-11 years with mean age 5.62±2.81 years. The largest group (30%, 9 out of 30 children) were from 2-3 years. Twenty children (66.7%) were males and 10 children (33.3%) were females.

FII activity was normal (80-110 %) before and after taking L-asparaginase. There was no obvious clinical evidence of thrombosis or bleeding.

The effect of L-asparaginase on FII activity (%) is shown in table 1. No significant effect of L-asparaginase was

observed on FII activity in the patients after taking the drug with mean activity of FII (%) = 97.83±1.71% as compared to mean FII activity before taking the drug which was 96.50 ±1.86% (P value =0.347) and when compared to mean FII activity of the control group which was 100 ±1.52% (P value =0.566).

Table 1: The effect of L-asparaginase on factor II activity.

Factor II (activity %)	ALL before	ALL after	Controls
Mean	96.50	97.83	100.00
Standard Deviation	10.18	9.35	8.30
Standard Error of Mean	1.86	1.71	1.52
Minimum	80.0	80.0	80.0
Maximum	120.0	110.0	110.0
Mode	100.0	100.0	100.0
Median	100.0	100.0	100.0
P value compared to control	**0.150**	**0.347**	**-**
P value compared to before		**0.566**	**-**

*Significant using Students-t-test for two independent means or paired t-test at 0.05 level of significance.

Discussion

L-Asparaginase, one of the cytostatics used during remission induction therapy for childhood ALL, is widely reported to impair the hemostatic system[10].

In this study L-asparaginase did not clinically show evidence of thrombosis on the patients who were included although the absence of the clinical evidence does not exclude the occurrence of thrombosis; as well as it did not significantly affect hemostasis based on the lab findings of FII activity assay that was tested before and after administration of the treatment.

This finding agrees with the findings of Inge M Apple, et al who run a study of 14 pediatric ALL patients with age range 11-16 years old. This study showed that the activation markers of thrombin generation and fibrinolysis did not change over time where blood sample was collected before each dose of L-asparaginase during the induction phase of that study period[11], but it disagrees with the results of Farrell K, et al[12] and also Gaurav Goyal, et al who retrospectively studied 548 ALL patients who were treated with L-asparaginase, that the median pretreatment antithrombin level of 120% was reduced to 59% in approximately half of the patients after the fourth dose of L-asparaginase[13].

Farrell K, et al found out in a prospective study of 45 ALL patients who were treated with L-asparaginase containing phase I induction protocol that anti-thrombin was reduced in 76% of patients who showed a significant reduction in thrombosis after receiving an AT replacement therapy[12].

Unaffected thrombin (FII activity) may be attributed to concomitant use of steroid that might lead to a rise in endogenous thrombin[14] resulting in a state of unstable balance between coagulation factors coagulation factors inhibitors[15] like antithrombin that was not measured in this study.

The possibility of subclinical thrombosis or bleeding though cannot be excluded since most thrombotic events induced by L-asparaginase are asymptomatic[5]. Especially those patients were not evaluated by imaging technique e.g. Doppler venography, echocardiography or MRI.

However thrombotic or bleeding events could be clinically observed if a larger group of ALL patients taking L-asparaginase was studied and for longer period of time.

In conclusion, the activity of factor II (thrombin) was not significantly affected by L-asparaginase in children with ALL.

References

1- Campana D, Hon Pui C. Childhood acute lymphoblastic leukaemia. In: Hoffbrand AV, Catovsky D, Edward GD, Tuddenham EG, Green AR, (editors). Postgraduate Haematology, Chapter 25, 6th ed. UK: Wiley–Blackwell Publishing; 2011. p.448-58.

2- Whitlock JA, Gaynon PS. Acute lymphoblastic leukemia in children. In: Greer JP, Foerster J, Rodgers GM, Paraskevas F, Glader B, Arber DA, et al (editors). Wintrbe's Clinical Hematology 12th ed. Philadelphia: Lippincott Williams & Wilikins publishing; 2009. Vol.1 - Chapter 80. p.1897-8.

3- Tubergen DG. The leukemias. In: Kliegman RM, Behrman RE Jenson HB, Stanton BF, (editors). Nelson Textbook of Pediatrics, 19th ed.**,** Philadelphia: Elsevier publishing; 2010 Chapter 487**,** p.1694 -6.

4- Hoelzer D, Gokbuget N, Ottmann O, et al. Acute Lymphoblastic Leukemia. ASH Education Book. 2002 vol. 2002 no. 1 p.162-192.

5- Hau C Kwaan. Double Hazard of Thrombophilia and Bleeding in Leukemia. ASH Education Book January 1, 2007 vol. 2007 no. 1 p.151-157.

6- Medlin SC, Perry MC. Hepatotxicity of chemotherapeutic agents. In: Perry MC (editor). The Chemotherapy Source Book. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2008 chapter 22. p.216.

7- Ziedins KB, Orfeo T, Everse SJ, Mann KG. Blood coagulation and fibrinolysis. In: Greer JP, Foerster J, Rodgers GM, Paraskevas F, Glader B, Arber DA, et al. Wintrbe's Clinical Hematology. 12th ed. Philadelphia: Lippincott Williams & Wilikins; 2009, Vol. 1, Chapter 20, p.546-55 .

8- Knorre DG, Kudryashova NV, Godovikova TS (October 2009). Chemical and functional aspects of posttranslational modification of proteins. Acta Naturae 2009; 1 (3): 29- 51.

9- Caen J, Larrieu MJ, Samama M, L'hémostase. Méthodes d'exploration et diagnostic pratique. Paris: L'Expansion scientifique,1975. Commercial kit used for FII assay (Deficient II substrate plasma for factor II assay REF 00745 / Diagnostica Stago).

10- Gatot D, Pringgardini K, Suradi R. Coagulation abnormality as a complication of L-asparaginase therapy in childhood lymphoblastic leukemia. PI [Internet]. 18Oct2016[Cited14Sep.2019];46(1):46-50. https://www.paediatricaindonesiana.org/index.php/paediatrica-indonesiana/article/view/854

11- Inge M Appel, Wim CJ Hop, Carla Van Kessel-Bakvis, Rolinda Stigter, Rob Pieters. L-asparaginase and the effect of age coagulation and fibrinolysis in childhood acute lymphoblastic leukemia. Thrombosis and Hemostasis 2008;100:330-7.

12- Farrell K, Fyfe A, Allan J, et al. An antithrombin replacement strategy during asparaginase therapy for acute lymphoblastic leukemia is associated with a reduction in thrombotic events. Leukemia Lymphoma 2016;57(11):2568-74.

13- Gaurav Goyal, Vijaya Raj Bhatt. L-asparaginase and venous thrombo-embolism in acute lymphocytic leukemia. Future Medicine 2015;11(17):2459-70.

14- CJ Majoor, MMS Sneeboer, A DE Kievit, et al. The influence of corticosteroids on hemostasis in healthy subjects. Journal of Thrombosis and Haemostasis 2016;14:716-23.

15- Appel IM, Hop WCJ, Pieters R. Changes in hypercoagulability by L-asparaginase: A randomized study between two L-asparaginase. Blood Coagulation and Fibrinolysis 2006; 17: 139-46.

IMJ 2020; 66(1):57-60.