ORIGINAL PROF-751

LYMPHOBLASTIC LEUKEMIA;
VARIATIONS IN BIOCHEMICAL PARAMETERS TOXICITY WITH THE TYPE OF INDUCTION TREATMENT

DR. M. ZAHOOR-UL-HASSAN DOGAR
MBBS, M.Sc (Hons), M.Phil(Pb)
Assistant Professor of Biochemistry,
Punjab Medical College Faisalabad.

DR. MUNIR A. SHIEKH, Ph.D
Chairman Department of Chemistry,
University of Agriculture Faisalabad.

DR. M. ASGHAR BAJWA, Ph.D
Assistant Professor of Biochemistry,
University of Agriculture Faisalabad.

Dr. M. Shoaib Akhtar, Ph.D
Dean Faculty of Veterinary Science,
University of Agriculture Faisalabad.

Dr. M. Farooque, MBBS
Senior Medical Officer
Govt. M.C Dispensory Noorpur, Faisalabad.

Mr. Muhammad Nawaz, M.Sc
College of Paramedics, Faisalabad.

Dr. Farkhanda Almas, MBBS
Deputy Director Technical
Population Welfare Department, Faisalabad

Correspondence:
Dr. M. Zahoor-ul-Hassan Dogar
Assistant Professor of Biochemistry
Punjab Medical College, Faisalabad.

ABSTRACT ... drzahoordogar@yahoo.com The present study was the continuation of the project in which two different treatment protocols were applied to evaluate the efficiency of each treatment. 42 total cases were taken divided equally into two groups. Group 1 was treated by conventional treatment DVP (Daunorubicin + Vincristine + Predinisolone). In 2nd Group DVP & L-asparginase enzyme was applied. The effect of two treatments on various biochemical parameters were studies by taking samples after each 0.5% months during induction therapy (3 months) and after induction therapy till 6 months. The results showed greater rise in blood ALT, ALP and amylase levels in Group II, as compared to the findings found in Group I. Similarly a relative rise in blood glucose, urea and bilirubin was also found in Group II as compared to Group I. It clearly indicates that after addition L-asparaginase although beneficial for induction therapy but resulted in deleterious effects development on different vital organs in the body. Its use should be strictly under control of a clinical biochemist so that its toxicity should not over balance its benefits.
Key Words: ALL, Acute Lymphoblastic Leukemia, DVP, Daunorubicin, Vincristine, Predinisolone, ALT, Alanine Transaminase, ALP, Alkaline Phosphatase, Induction Treatment.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disorder resulting from the clonal proliferation of lymphoid precursors with arrested maturation[1]. Most

common malignancy presents in children (about 66%)[2] and of course in majority of the children[3], this is the malignancy which respond to treatment. About 3000 - 5000 peoples in USA hear this news each year that they are suffering from acute lymphoblastic leukemia[2]. Median age at diagnosis is usually about 12 years[4,5]. Showing a bimodal distribution with peak age 2-5 years and again at 50-65 years[6,7].

Although the prognosis of the patients with ALL has improved markedly during the last decades, newer strategies including more dose intensive therapy, search for new drugs and more target specific therapy are needed to improve the current cure rate[8]. The conventional drugs used for treatment of Acute Lymphoblastic Leukaemia (ALL) are daunorubicin, vincristine and prednisolone. L-asparaginase (L-asnase) is used for synergistic activity, supported by radiotherapy and/or methotrexate for metastasizable places like central nervous system and testis etc[6,7,9,10].

L-asparaginase is a potent anti-cancer agent in ALL because this impairs asparagine synthesis. This is lethal to cells that cannot synthesize the essential amino acid, asparagine. Hypersensitivity and pancreatitis are documented contraindications of this drug use, an other common side effects include bone marrow depression, hyperglycemia, hepato-toxicity and bleeding from different sites[7].

Keeping in view the above facts, the present project has been designed with the following objectives;

1. To evaluate the role of conventional chemotherapy without L-asparaginase on different biochemical parameters in acute lymphoblastic leukaemia in Faisalabad.
2. To evaluate the additional role of L-asparaginase enzyme with conventional chemotherapy on different biochemical parameters in acute lymphoblastic leukaemia in Faisalabad.

The effects of the above two treatment protocols on different biochemical parameters which are helpful for evaluation of different toxic side effects on the body.

MATERIALS & METHODS

Forty two patients were included in the study. The subjects were divided equally into two groups, for induction of remission therapy. To group I, to study and review the conventional chemotherapy and group II conventional with added L-asparaginase 5000 IU twice per week were given. Seven patients could not complete the trial due to various reasons and three were died, 2 in group one and 1 in group 2. So 32 (16 in each group) could complete the study. Ten normal subjects were taken for comparison, having age, sex and socioeconomic status matched with experimental group. Subjects suffering from acute lymphoblastic leukaemia were selected from out and in-patient departments of govt. And private sector hospitals, including those cases being referred by general sector hospitals for this purpose. The completely normal healthy subject were selected from the local population.

As the study was on human subject so prior permission from the local ethical committee was taken to start the project. All the subjects were briefed about the project at start, their consent was taken, the complete history and clinical check up was done to see any other abnormality. Ten ml blood samples were taken and were immediately transferred to test tubes, to separate serum, for estimation of various biochemical parameters[12].

Biochemical Parameters

1. Serum glucose
2. Serum urea
3. Serum bilirubin(Direct, indirect and total)
4. Serum Alanine Transaminase (ALT)
5. Serum Alkaline phosphatase (ALP)
6. Serum Amylase

The project was completed as three months duration of treatment for induction of remission with or without L-asparaginase therapy while biochemical tests were performed after each 0.5 months initial 3 month (induction therapy) and later up to 6 months

(maintenance therapy) in order to see the prognosis of disease and development of any side effects by the treatment. The difference between the means of the different parameters before and after drug administrations in the experimental and control groups was worked out. Data collected was analyzed by appropriate statistical analysis[11,13].

RESULTS

The biochemical parameters determined in group I were serum glucose, urea, bilirubin and enzyme levels of ALT, ALP and amylase. The serum glucose, urea, bilirubin, direct, indirect and total levels were 92, 42, 0.4, 0.7 and 1.1 mg/dL respectively at zero time period. While these levels were 90, 51, 0.9, 1.1 and 2.0 mg/dL at 3 months and 95, 46, 0.6, 0.9 and 1.5 mg/dL after six months. The enzyme levels of ALT, ALP and amlylase were 44, 64, and 40 IU/L at zero period and 68, 100 and 52 IU/L at 3 months and 42, 62 and 45 IU/L after six months.

The results are shown in Table-I. Similarly the biochemical parameters determined in group II the serum glucose, urea, bilirubin direct, indirect and total levels were 96, 46, 0.4, 0.9 and 1.3 mg/dL respectively at zero time period. While these levels were 102, 62, 0.8, 2.0 and 2.8 mg/dL at 3 months and 120, 60, 0.6, 1.2 and 1.8 mg/dL after six months duration. The serum enzyme levels of ALT, ALP and amylase were 48, 64 and 40 IU/L at zero period and 120, 89 and 108 IU/L at three months and 92, 68 and 66 IU/L after six months of treatment. The result are shown in Table-II. The results were compared between different treatment groups and time periods, by applying ANOVA test. The treatment group interactions with different time periods were also calculated by the same test. The results of this comparison is given in Table-III which clearly indicates that how these groups behave in different time periods.

In order to evaluate the side effects developed (our primary aim) on different organs of the body, various biochemical parameters were estimated which showed that in the experimental group 1 (without L-asparaginase) out of 16 patients nobody showed pancreatitis while one showed renal, 2 showed hepatotoxicity and nobody showed abnormal glucose levels. Similarly these values in the experimental group 2 (with L-asparaginase) showed that out of 16 patients who completed, 1 patient showed pancreatitis and 2 developed renal and 3 showed hepatotoxicity.

Table-I. Different biochemical parameters before and after chemotherapy without L-asparaginase.

Parameters	Units	Months						
		Start	1.0	2.0	3.0	4.0	5.0	6.0
1.Serum Glucose	mg/dL	92	110	105	90	100	90	95
2.Serum Urea	mg/dL	42	60	58	51	48	44	46
3.Serum Bilirubin Direct	mg/dL	0.4	0.6	0.7	0.9	0.4	0.5	0.6
Indirect	mg/dL	0.7	1.2	1.2	1.1	1.4	1.0	0.9
Total	mg/dL	1.1	1.8	1.9	2.0	1.8	1.5	1.5
4. Serum ALT	IU/L	44	56	42	68	52	40	42
5. Serum ALP	IU/L	64	62	64	100	90	72	62
6. Serum amylase	IU/L	42	50	52	52	74	46	45

Table-II. Different biochemical parameters with DVP with L-asparaginase therapy.

Parameters	Units	Months						
		Start	1.0	2.0	3.0	4.0	5.0	6.0
1. Serum Glucose	mg/dL	96	146	140	102	90	98	120
2. Serum Urea	mg/dL	46	62	64	62	66	58	60
3. Serum Bilirubin Direct	mg/dL	0.4	0.8	1.0	0.8	0.6	0.4	0.6
Indirect	mg/dL	0.9	1.2	1.6	2.0	1.5	1.2	1.2
Total	mg/dL	1.3	2.2	2.6	2.8	2.1	1.6	1.8
4. Serum ALT	IU/L	48	70	82	66	72	56	92
5. Serum ALP	IU/L	64	90	82	89	80	80	68
6. Serum amylase	IU/L	40	78	102	108	76	84	66

Table-III. Comparison among different parameters in treatment groups and time periods by applying ANOVA tests

Parameters	F. VALUES WITH SIGNIFICANCE		
	Treatment Groups (I & II)	Time periods (1-13)	Interactions (groups x period)
Glucose	19.246**	0.1821 (NS)	0.2241 (NS)
Urea	44.753**	17.603**	0.910 (NS)
Bilirubin Direct	18.384**	1.267*	0.609 (NS)
Bilirubin Indirect	66.043**	2.299**	0.780 (NS)
Bilirubin Total	84.719**	3.461**	0.886 (NS)
ALT	63.570**	26.026**	2.269**
ALP	40.045**	8.910**	1.012*
Amylase	15.043**	0.348 (NS)	0.339 (NS)

**Significant, **Highly Significant, NS = Non significant*

Table-IV Comparison of toxic effects in different treatments

No.	Toxic Effects	DVP without L-asparaginase	DVP with L-asparaginase
01	Impared Glucose tolerance	-	1
02	Diabetes Mellitus	-	1
03	Urea rise in blood	1	2

04	ALT rise in blood	2	3
05	Bilirubin rise in blood	1	3
06	Amylase rise in blood	-	1
07	Hyper-sensitivity Reactions	-	1(mild)

There was one patients who developed diabetic glucose blood levels and one had blood glucose level 130mg/dL (between 120-140mg/dL) showing an impaired glucose tolerance. The comparison of toxic effects produced are shown in table-IV.

DISCUSSION

These results are comparable to another study[17], stated that L-asparaginase is a foreign protein so severe dose limiting hypersensitivity reaction are possible. In that study 78% showed complete remission after 35 days period combination therapy. Anaphylaxis did not occurred during treatment. Mild urticaria and mild local allergic reaction occurred in 5 patients but did not cause discontinuation of therapy.

The incidence of hyperglycemia and pancreatitis was minimal. Only one patient was hypersensitive to L-asparaginase on enrollment, so single agent activity was documented in ALL by them.

In another study[15], 14 patients developed hyperglycemia during induction therapy of ALL with L-asparaginase, prednisolone, vincristine and daunorubicin. Hyperglycemia developed after a mean of 5 doses. Seven of 14 patients had neutropenic related infections episode. Hyperglycemia resolved in all patients within 12 days and 2 patients died of neutropenic septicemia.

During the re-induction therapy with the same drug only one patient developed hyperglycemia. They recommended close monitoring during L-asparaginase therapy for hyperglycemia because it enable prompt recognition and early correction, preventing delay in therapy of acute lymphoblastic leukaemia. Similarly in another study[16], two cases of hyperglycemia were reported in childhood acute lymphoblastic leukaemia treated with L-asparaginase.

In another study experiment[17] the most frequent side effects were pancreatitis, hyperbilirubinemia, diabetes mellitus, diarrhoea and hypofibrinogenemia were seen with L-asparaginase therapy.

Similarly Alverez and Zimmerman 2000[18], reported severe toxicity in 7 children showing an increased amylase levels in blood. Similar sort of study[19] concluded that 14 patients developed hyperglycemia during combination induction therapy with L-asparaginase. They reported that hyperglycemia usually developed after 5 doses and resoled within 12 days of discontinuation of L-asparaginase. therapy.

Our findings are consistent with the already stated toxic side effects related with L-asparaginase therapy. So hypersensitivity reactions, pancreatitis, hyperglycemia and hepato-renal toxicity are most common and frequently occurring side effects of L-asparaginase therapy.

However, a mild rise in blood glucose, urea, bilirubin and serum ALT and Amylase levels in treatment group I without L-asparaginase indicates that these may be related with the increased metabolic demands of the body with combination of anti-cancer agents and the body in unable to cope with this large load on the excretion of body metabolites as indicated by excessive cellular break down in the body by anti neoplastic agents. This is enhanced to a greater extent by the addition of L-asparaginase enzymeas indicated in group II.

CONCLUSION

The addition of L-asparaginase enzyme in injection form may be resulted in development of various side

effects. These are commonly hyperglycemia. renal, hepatic and pancreatic toxicity. So addition of L-asparaginase enzyme with conventional chemotherapy should be monitored closely in order to produce maximum benefits and minimum side effects which can be obtained by proper and controlled supervision of an efficient Oncologist in association with an expert clinical Biochemist and Hematologist. A team work is necessary for the effective management of acute lymphoblastic leukaemia.

REFERENCES

1. Sawyers CL Deny CT, Witte ON. **Leukema and the disruption of normal haematopoisis.** Cell 1991, 64: 337-50.

2. Brinker H. **Population based age and sex distribution rates in 4 main types of leumemia.** Scand J Haematol 1991, 29; 241-49.

3. George SL, Aur RTA, Maure AM and Simone JV. **A repraisal of the results of stopping therapy in childhood leukemia.** New Eng J Med . 1979;300: 269-73.

4. Lukens JN. **Acute lymphoplastic leukemia in Lec** GR, Bithel TC, Foerster J, Kethenstw, Lukens J N (editors) Winthrobe Clinical Haematology. 9ed Philadelphia. Lee and Febiger 1993; pp1892-1919.

5. Poplac DG. **Clinical manifestation of ALL.** Med Pedia. On col 1991;14:166-72.

6. Barow OVSKY A , Myes MH. **Cancer incidence and survival in patients 65 years of age and older.** Cancer J Clin 1986; 36: 26-41.

7. Sandler DP. **Epidemiology and etiology of acute leukemia; and update.** Leukemia 1992; 6(4Supp): 3-5.

8. Cortes JE and HM Kantar Jian. **Acute lymphoblastic leukemia; a Review.** Cancer 76; 2393-2417.

9. Aziz Z. **Haematological malignancies. The editorial.** Pak. J. Med. Res. 1989: 28:(4) 221-22.

10. McVie JG. **Chemotherapy of malignant diseases. Clinical pharmacology 24th Edition.** London. Bailliere Tendall. 1989: 496-525.

11. Alfieri DR.; **PEG asparaginase.** Pediatrics Nutrition 1995: 21: 471-474.

12. Dacie JV and Lewis SM. **Practical haematology 8th Edition. Edinburg;** Churchil Living Stone. 1996: 1-351.

13. Muhammad F. **Analysis of various in; Statistical Methods and Data Analysis. Faisalabad;** Kitab Merkiz. 1994: 273-304.

14. Larson RA, RK Dodge, CP Burns, EJ Lee, RM Stone, P Shulman, D Duggan. **A five drug remission, induction regime with intensive consolidation for adults with ALL.** Blood. 1995: 85(8): 2025-37.

15. Pogliani EM, M. Parma, I. Baragett, G. Mustarda, F. Fivolta, P. Maffe, G. Corneo. L. **Asparaginase in acute lymphoblastic leukaemia treatment.** Acta. Heamtol. 1995: 93: 5-8.

16. Ettinger LJ, J Kurtzberg, PA Voute, H Turegen, SL Halpern. **An open label study of PEG-Asparginase for treatment of ALL.** Cancer. 1995: 75: 1176-81.

17. Nagura E, Kimura k, Yamada K, Ota K, Mackawa T, Takaku F and Uchino H. **Nation wide randomized comparative study of doxorubicin, Vincristine prednisolone combination therapy with and without L-asparaginase for adult ALL.** Int. J Haematol. 1994; 33: 359-65.

18. Alvarez JA and Zimmerman G. **Pegasparaginase induced pancreatitis.** Med. Paedia. Oncol. 2000; 34: 200-205.

19. Iyer RS, Rao SR, Pai S, Adcani SH and Magrath IT. **L-asparaginase related hyperglycemia.** Indian J. Cancer. 1993; 30: 72-76.