LIPID PROFILES IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA ON L-ASPARAGINASE THERAPY

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ABSTRACT

Background: **Abnormal blood lipid profiles have been associated with many malignant diseases especially in children with acute lymphoblastic leukemia on L-asparaginase therapy**

Objective: **To investigate the frequency and clinical significance of altered lipid profiles in children with Acute lymphoblastic leukemia (ALL) on L-asparaginase therapy**

Patients and Methods: A prospective study was carried out between February 2008 till January 2009 on a total of 50 patients with recently diagnosed and non-treated acute lymphoblastic leukemia who were admitted to pediatric oncology unit at Basrah Maternity and Children hospital. Fasting blood for lipid profiles [cholesterol (CH), Triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein(LDL), very low density Lipoprotein (VLDL)] were obtained from 30 children with Acute Lymphoblastic Leukemia at diagnosis, during induction therapy with L-Asparaginase and after induction without L-Asparaginase, their ages range between (1-15) years and 20 patients were excluded because of incomplete information (non compliance).

Results: **Thirty patients with Acute Lymphoblastic Leukemia (ALL) were studied, 20(66.7%) males and 10(33.3%) females. An altered lipid profile was observed during therapy with L-asparaginase especially (CH,TG), the means (222.3±62.9 mg/dl, 223.1±88.31 mg/dl) respectively were higher than the level before the initiation of therapy (158±36 mg/dl, 119.4±57.3mg/dl) and after induction (139.8±61.5 mg/dl, 96.2±30.7 mg/dl) and these were statistically highly significant (P-value <0.001) while no significant changes regarding other types of lipid profile. This study showed no significant changes of lipid profiles in relation to morphological type, risk of leukemia, sex, age and body mass index (BMI), before, during and after therapy with L-Asparaginase.**

Conclusion: **Hyperlipidemia especially cholesterol and triglyceride levels were significantly higher during treatment with L -Asparaginse and were reversible after drug discontinuation but showed no significant correlation with age, BMI, risk group and morphological types.**

INTRODUCTION

Abnormal blood lipid levels have been associated with various forms of cancer including acute lymphoblastic leukemia (ALL), but a consistent profile has not emerged.[1-3] Asparaginase is a bacterial enzyme, which is an effective drug in childhood acute lymphoblastic leukemia protocols and it is used during the induction and intensification phase, it depletes the blood pool of asparagines, nonessential amino acid. Malignant lymphoid cells, in contrast to most of the healthy tissue cells, are unable to synthesize asparagine when there is no nutritional supply of this amino acid so that the depletion of the systemic pool of asparagine will lead to malignant cell death.[4] The native (unmodified) forms of L-asparaginase are derived from *Escherichia coli* or *Erwinia carotovora* and are administered intravenously or intramuscularly. The intramuscular route is more commonly used in children because of lower risk of severe allergic reaction. Conjugation of polyethylene glycol to E-coli asparaginase (PEG-asparaginase) lowers the immunogenicity of this foreign protein and prolongs the drugs half-life, allowing for less frequent administration.[5] Although L-asparaginase is an effective anti-leukemia agent, it is also associated with side effects occurring either in a dose or time dependent fashion or as hypersensitivity reactions.[6] The most frequently encountered toxicities in L-asparaginase containing regimens are allergy (20%), thromboembolic events (2-11%), severe pancreatitis, hyperglycemia, and encephalopaty. [7,8] A possible adverse reaction is an elevation in plasma triglyceride (TG) concentration which is reported to occur in up to 10% of treated children treated.[9] The

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mechanism responsible for the hypertriglyceridemia is unknown, but a relationship with lipoprotein lipase (LPL) has been suggested which is an endothelial-bound enzyme responsible for triglyceride hydrolysis of circulating TG-rich lipoproteins, it is the rate-limiting enzyme for the removal of TG from circulation.[10] So the fasting plasma concentration of TG correlates with the activity of LPL[10,11], and L-asparaginase therapy may reduce synthesis of lipolytic enzymes (such as hepatic triglyceride lipase).[12] This study was carried out to determine whether there are abnormal blood lipid profiles that are specific for acute lymphoblastic leukemia and its treatment especially L-Asparaginase and its relations to age, sex, risk group and morphological types.

PATIENTS AND METHODS

A prospective study was carried out between February 2008 till January 2009. A total of 50 patients with recently diagnosed and non-treated acute lymphoblastic leukemia who were admitted to pediatric oncology unit at Basrah Maternity and Children hospital were included. All patients had a comprehensive diagnostic work up for accurate diagnosis stratified by risk group [based on white blood cell count, age, and the presence of central nervous system disease] and morphological classifications depend on morphology of blast cell [L1,L2,L3]. Their ages ranged between (1-15 years).There were 30 males an 20 females. History and physical examination were carried out including vital signs, measurements like body weight, height or length, BMI, and systemic examination. All these Patients had no family history of hyperlipidemia or history of a premature cardiovascular or cerebrovascular event (<55 years of age). Twenty patients were excluded because of incomplete information (non compliance). Thirty out of 50 patients had received induction chemotherapy in which prednisolone and vincristine were given together with L-Asparaginase (derived from *Escherichia coli*). Doxorubicin was also added to the treatment for high risk patients. Blood samples were obtained after an overnight fast for children whose ages were more than two years and 2-4 hours for children less than two years. The determination of serum lipids was done before, during induction of remission (where L-Asparaginase treatment was given for 3 or 4 consecutive days with a one week interval) and two weeks after completion of L-asparaginase therapy. Blood specimens were collected for lipid testing and were analyzed on the same day. Serum cholesterol (CH) and triglycerides (TG) were measured enzymatically on the Johnson and Johnson, warren, NJ. Serum high density lipoprotein cholesterol (HDL-C) was measured enzymatically on the same instrument, very low density and low density lipoproteins (VLDL and LDL) using dextran sulfate and magnesium sulfate as the precipitin reagent. LDL was calculated from these values using the formula of Friedewald et al. [13] Results were categorized according to hospital laboratory norms. Normal triglyceride was defined as a serum concentration of less than 110 mg/dl.[14] Cholesterol levels were categorized as desired less than 170 mg/dl, border line 170-199; and high≥200. [15] Lipid profiles were correlated with age, sex, BMI, risk groups, and morphological subtypes of leukemia before, during and after L-asparaginase therapy.

Statistical analysis was done to find the p. value using SPSS version 15, student's t test and pearson correlation were performed to determine differences between groups at diagnosis, during and after treatment. A value of $P<0.05$ was considered to be significant.

RESULTS

The age and sex distribution of studied cases hyperlipidemia is presented in (Table-1). Twenty were males and the rest were females. More than half of children aged 1-5 year.

Table 1. Age and sex distribution of patient with hyperlipidemia.

Age	Sex					
	Male		Female		Total	
	N	%	N	%	N	%
1 – 5	10	50%	6	60%	16	53.3%
6 – 9	6	30%	2	20%	8	26.7%
10 – 15	4	20%	2	20%	6	20%
Total	20	100%	10	100%	30	100%

Table (2) Shows the mean Lipid profile concentration in treated patients with L-Asparaginase, the mean Ch, TG concentration before treatment were158 mg/dl, 119.4 mg/dl respectively, during 222.3 mg/dl 223.1 mg/dl respectively and after 139.8 mg/dl 96.2 mg/d respectively were significantly higher during therapy than before and after (P<0.0001), and significant low LDL was found after therapy.

Table 2. Lipid profile before, during and after L.Asparaginase therapy.

Lipid profile	N	CH Mean±SD	P.	TG Mean± SD	P.	Hdl Mean±SD	P.	Ldl Mean±SD	P.	Vldl Mean±SD	P.
Before therapy	30	158 ±36	0.0001	119.4± 57.3	0.0001	± 41.0 16.4	0.5	94.2 ± 29	0.9	27.5 ± 15.5	0.14
During therapy		222.3 ± 62.9		223.1 ± 88.32		39.2 ± 11.3		94.1 ± 39.7		36.8± 19.7	
After therapy	30	139.8 ± 61.5	0.0001	96.2 ± 30.7		46.0± 19.4	0.18	71.3± 37.2	0.03	31.0 ±31.9	0.3

Table (3) Shows correlation between age and lipid profile before, during and after treatment. There was found no significant correlation between hyperlipidema before, during and after therapy with L-asparginase and the ages of patients.

Table 3. Correlation between Age and lipid profile before, during and after L.Asparaginase therapy.

Age pearson correlation (No. of patients 30)		CH	TG	HDL	LDL	VLDL
Before induction	Pearson correlation	-.267	-.032	-.020	-.196	-.210
	Sig.	.154	.868	.917	.300	.266
During induction	Pearson correlation	-.373	-.178	-.007	- .475	- .450
	Sig.	.042	.348	.972	.008	0.13
After induction	Pearson correlation	-.026	-.276	-.103	-.099	-.358
	Sig.	.890	.140	.590	.601	.052

Table (4) Shows correlation between lipid profile with the risk group of leukemia before, during and after treatment. There was found no significant correlation between risk group and Lipid profile.

Table 4. Lipid profile in relation to the risk group of leukemia before, during and after L. Asparaginase therapy.

Type of risk		N	CH. MEAN SD	P.	TG MEAN SD	P.	HDL MEAN SD	P.	LDL MEAN SD	P.	VLDL MEAN SD	P.
Before Therapy	S. Risk	8	175.1 ± 47.3	0.4	139.8 ± 76	0.4	36.5 ± 16.7	0.	90 ± 23.6	0.3	31.2 ± 12	0.
	H. Risk	22	151.8 ± 29.8		112 ± 42.7		42.7 ± 16.4		95.7 ±32.1		26.3 ± 16.3	
During therapy	S. Risk	8	236.8 ± 102	0.1	221.7 ± 109.6	0.2	38.6 ± 72	0.	86 ± 39.6	0.5	23.1 ± 17.2	0.
	H. Risk	22	217 ± 43.3		227.8 ± 72		32.5 ± 11.9		97 ± 40.2		39.1 ± 21.4	
After therapy	S. Risk	8	147.5 ± 22.1	0.3	89.6 ± 38.4	0.02	475 ± 18.4	0.	70.7 ± 32.1	0.7	18.2 ± 8.	0.
	H. Risk	22	132.4 ± 31.9		92.5 ± 27.2		44.3 ± 19.2		76.7 ± 40.6		35.2 ± 33.6	

(Table-5), Shows the relation between lipid profile and patient's sex before, during and after treatment. There was no significant relation between sex and lipid profile.

Table 5. Lipid profile in relation to sex before, during and after L-Asparaginase treatment

Sex		No.	CH. Mean ±SD	P.	TG ± Mean	P.	HDL Mean ±SD	P.	LDL Mean ±SD	P.	VLDL Mean ±SD	P.
Before Therapy	Male	20	154.2 ± 22.5		118 ± 53.3	0.2	42 ±18.4	0.3	91.8 ± 25		26.5 ± 12.6	0.2
	Female	10	165.7 ± 54.8		122.30 ±67.5		39.2 ±12.2		99 ± 38		29.8 ± 20.2	
During therapy.	Male	20	225.6 ± 70	0.9	223.2 ± 81.7	0.7	385 ± 10.9	0.9	96.9 ±413	0.6	36.8 ± 19.7	0.4
	Female	10	215.6 ± 46.5		232.1 ±85.5		40.7 ±12.5		88.5 ±37.8		31. ±24	
After therapy	Male	20	141.9 ± 31.3	0.2	88.4 ± 20.3	0.5	48.8 ± 20.3	0.2	69.5 ± 36.9	0.7	34.8 ± 7.9	

(Table-6), shows the correlation between lipid profile and BMI before, during and after treatment. There was no significant correlation between BMI and lipid profile.

Table 6. Correlation between BMI and lipid profile before, during and after L-Asparaginase therapy

		CH	TG	HDL	LDL	VLDL
Before	Pearson correlation	- 0.121	-0.177	- 0.037	- 0.331	- 0.039
	P value	0.5	0.3	0.8	0.07	0.8
During	Pearson correlation	- 0.138	-0.052	- 0.163	- 0.146	- 0.055
	P value	0.4	0.7	0.3	0.4	0.7
After	Pearson correlation	- 0.183	-0.022	- 0.072	- 0.338	- 0.027
	P value	0.3	0.9	0.7	0.06	0.8

(Table-7), Shows no significant relation between morphological types of acute lymphoblastic leukemia (L1,L2) and lipid profile before, during and after therapy.

Table 7. Lipid profile in relation to morphological subtype of leukemia before, during and after L.Asparaginase therapy.

Morphological Type		N	CH. MEAN SD	P.	TG MEAN SD	P.	HDL MEAN SD	P.	LDL MEAN SD	P.	VLDL MEAN SD	P.
Before Herapy	L1	9	169.3 ± 49	0.2	147.2 ± 71.6	0.0	32.3 ± 13	0.2	91.8 ± 24.3	0.44	30.1 ± 11.6	0.3
	L2	21	153.1 ± 28.8		107.5 ± 47		44.8 ± 16.3		95.1 ± 32.4		26.5 ± 16.7	
During Therapy	L1	9	236.0 ± 97.	0.1	221.1 ± 102	0.4	43.4 ±13.6	0.1	83.3 ±39.9	0.4	35.2 ± 23.1	0.7
	L2	21	216.4 ± 43		228.3 ±74		37.4 ±10		98.7 ±39.7		34.7 ±21.1	
After Therapy	L1	9	147.8 ± 29.2	0.7	92.3 ±33.9	0.4	40.4 ± 16.0	0.4	68.1 ±34.7	0.7	32.3 ±37.1	0.8
	L2	21	131.5 ± 29.4		91.4 ± 28.9		47.2 ±12.7		77.9 ± 4.		29.8 ± 27.2	

DISCUSSION

Lipid abnormalities have been described in children with Acute lymphoblastic leukemia (ALL) who have been treated with L-asparginase. In the current study hyperlipidemia was estimated to be 65.2% of patients with ALL during asparginase containing regimen. Similar results were observed by Ridola, et al,[16] while Parsons Sk[17] noticed hyperlipidemia only in 19% of patients with leukemia. The pathogentic mechanism of hyperlipidemia seems to be due to an increase in endogenous synthesis of VLDL and to a decreased lipoprotein Lipase activity with consequent decreased removal of triglycerides from plasma.[18] In this prospective study the mean serum cholesterol was significantly higher during therapy with L-asparginase as compared with the levels before therapy, similar results were observed by Naik et al[19]. This study also showed a significantly increased levels in TG, during therapy with L. asparaginase which was in agreement with finding of Mousolino *et al* [20] and Florenza, et al. [21] The explanation of low cholesterol levels before therapy is due to increase cholesterol synthesis and accumulation of cholesterol esters in tumor tissues and due to increase catabolism after therapy so it is considered as possible prognostic factor in malignancies as suggested in some studies.[22,23] The present results prompted the hypothesis that the hypertriglyceridemia is induced by a suppression of lipoprotein lipase activity as a result of an increase in the apoCIII/apoCII ratio since apoCII is an important co- factor for lipoprprotein lipase and an excess amount of apoCIII inhibits its activity. [24,25] Another study was done by Halton et al, [26] showed extremely elevated TG levels during combination therapy with L-asparaginase and corticosteroid, as corticosteroids and L-asparaginase are active antineoplastic agents most commonly used together in the remission induction phase of ALL that were not observed during therapy with steroid without L-asparaginase in children with solid tumors as there was no role of L-asparagiase in their protocol of therapy and suggested that L-asparaginase lead to specific alterations and may provide insight into the toxicity associated with the drug. Among the

numerous effects of steroids is their influence on lipid metabolism, yet no consistent changes in plasma lipid have been reported after induction, the L-asparginase was discontinued and lipid profile was performed with rapid resolution of hyperlipidemia and there was no recurrence with next pulse of steroid treatment. [26]

In the present study, plasma LDL was siginficantly increased during therapy similar result was observed by Patel MM, et al[27] and the explanation of this, that these individuals have deficient or defective LDL receptors so they remove plasma LDL at much lower rate and have considerably elevated levels.[28] On contrary ,in another study by parsons et al[17] observed a decrease in the ratio of LDL to apoB (which may indicate a shift to smaller, denser LDL particles) during treatment with L–asparaginase that reversed during maintenance therapy without L-asparaginase. This study showed no significant change of lipid profiles in relation to morphological type, risk of leukemia, sex, age and BMI before, during and after therapy with L-asparaginase, similar results were observed by other studies.[1,29,30]

In conclusions, hperlipidemia due to L-asparaginase is reversible after drug discontinuation and is rarely associated with severe complications therefore antileukemic treatment should not be modified because of hyperlipidemia

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