Effect of maintenance therapy for childhood with acute lymphoblastic leukaemia by combination of methotrexate and 6–mercaptopurine on the liver.

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الخلاصة

سبعة من أصل 30 طفلاً يعانون من الليوكيميا الحادة (ALL) والذين يتلقون علاجاً متكوناً من 6MP يومياً عن طريق الفم و MTX أسبوعياً أخذوا يعانون من تلف الخلايا الكبدية وإنسداد قناة الصفراء مع إرتفاع غير طبيعي في مستويات انزيمات الـGPT,GOT والدالة على تلف خلايا الكبد والـALP,GGT,والـTSB الدالين على إنسداد قناة الصفراء.
أما الثلاثة والعشرون مريضاً الآخرين فقد كانت مستويات الـTSB,GGT,ALP ضمن المعدل الطبيعي عدا عن ارتفاع نسبة الـGPT,GOT في 13 مريضاً منهم
الاستعمال الطويل الأمد للـMTX (20ملغم/م^2/اسبوع) والـMP, (75ملغم/م^2/اليوم) في علاج ليوكيميا الدم الحاد أدى إلى تلف خلايا الكبد بواسطة الـMTX وإنسداد قناة الصفراء بواسطة الـ6MP .

ABSTRACT

Seven of 30 children with acute lymphoblastic leukaemia (ALL) receiving maintenance therapy (MT) consisting of daily oral 6–mercaptopurine (6MP) and weekly methotrexate (MTX) developed both hepatocellular destruction and intrahepatic cholestasis (with abnormally elevated levels of serum aminotransferases enzymes GOT, GPT that mainly indicate liver cell destruction, and alkaline phosphatase, gammaglutamyl transferase enzymes, as well as total serum bilirubin that mainly indicate biliary tract disorder). In the remaining 23 patients, the serum levels of alkaline phosphatase (ALP), gammaglutamyl transferase (GGT) enzymes, and total serum bilirubin (TSB) . were within the normal reference ranges for these parameters of the liver function tests while 13 patients. have abnormally elevated serum glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transamiase (GPT). The long–term use of MTX (20 mg/m2/week) and 6MP (75 mg/m2/day) during the MT for childhood ALL may lead to the development of hepatocellular destruction mainly induced by MTX, and intrahepatic mainly induced by 6MP.

INTRODUCTION :

About 80% of children with leukemia have acute lymphoblastic leukemia.[1]. It represents the malignant clonal proliferation of immature lymphoid precursors (blasts) that replaces the bone marrow and emigrates into peripheral blood and infiltrate other tissues and organs. Over the last 2 decades, multi-drug protocols, early and late treatment intensification, central nervous system prophylactic chemotherapy, and maintenance therapy have increased the event free survival rate in childhood ALL to 70 –75%[2,3]. However, the core of MT consisting of daily oral 6MP and weekly. has remained almost unchanged .Both 6MP and MTX are anti–metabolites: 6MP is a purine analogue. They affect the synthesis of RNA and DNA precursors and the functioning of normal cells [4]

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PATIENTS AND METHODS:

Thirty children (19 boys and 11 girls) with ALL under MT consisting of daily oral 6MP (75mg/m2/day) and weekly oral MTX (20mg/m2/week) for durations ranged between 5-.36 months. selected from ALMansour Pediatric. Hospital Their ages between (3-14) years median ages,(8.6) years They were divided according to the duration of MT received, into 4 groups (Table-1).

Table (1)

Group	No.	Duration of MT (months)	Age (years)	Male	Female
I	7	Up to 6	4 – 9	2	5
II	10	10 – 18	3 – 13	8	2
III	8	20 – 28	5 – 14	5	3
IV	5	30 – 36	6 – 12	4	1

Ten children (5 boys and 5 girls) with age ranged from 3-14 years with ALL who were newly diagnosed with the disease, were selected before starting the treatment protocol. This group of patients was considered as a baseline for this study. A group of 16 healthy children (9 boys and 7 girls), their ages ranged from3-15 years considered as controls.

Venous blood samples were collected and the biochemical liver function tests; serum transaminases enzymes (GOT, GPT), alkaline phpsphatase enzyme (ALP), gamma –glutamyl transferase enzyme (GGT), total serum protein (TSP), serum albumin (ALB), and total serum bilirubin (TSB) levels, were measured.

Statistical analysis were performed by using one way analysis of variance ; ANOVA–test and t–test for unpaired data in comparison between the baseline and control groups, and between the ALL patients under MT and the control subjects. $P<0.05$ were considered to indicate significance.

RESULTS:

There was insignificant difference between the baseline group (ALL patients before starting treatment) and the control group ($P>0.05$) in respect to the mean values of all parameters of the liver function tests involved in this study (Table-2).

Total serum protein, albumin for the 30 patients with ALL under MT., and were within the normal reference range for each one (Table-3).

Table 2 . Liver function tests measured in sera of control and baseline subjects.

Parameters	Control Subjects	Baseline Subjects	P–value	Level of Significance
	Means ± SD	Means ± SD		
S.ALB (mg/dl)	3.869 ± 0.36	3.841 ± 0.22	> 0.05	N.S.
TSP (mg/dl)	6.669 ± 0.519	6.631 ± 0.54	> 0.05	N.S.
TSB (mg/dl)	0.531 ± 0.2	0.630 ± 0.17	> 0.05	N.S.
S.GGT (U/L)	12.763 ±3.377	13.96 ± 2.547	> 0.05	N.S.
S.GOT (U/L)	8.125 ± 2.125	9.5 ± 2.718	> 0.05	N.S.
S.GPT (U/L)	8.719 .±2.695	10.1 .± 1.853	> 0.05	N.S
S.ALP (K-KU/dl	13.688.± 2.3	13.9 .± 2.33	> 0.05	N.S

Significant < 0.05
Not significant > 0.05
Normal value in serum:
GOT, GPT: up to 12 U/L.
ALP: children 10–20 kind and king unit/dl.
GGT (measured at 30°C): male 8–33 U/L, female 7–29 U/L.
TSB: 0.1–1.2 mg/dl.

Table 3 . TSP and ALB. for patients with ALL under MT and control subjects

Group	Parameters	Means ± SD		P–value	Level of Significance
		ALL patients under MT	Control subjects		
I	TSP (gm/dl)	6.74 ± 0.51	6.669 ± 0.52	> 0.05	N.S.
	ALB (gm/dl)	4 ± 0.13	3.869 ± 0.36	> 0.05	N.S.
II	TSP (gm/dl)	8.28 ±0.43	6.669± 0.52	> 0.05	N.S.
	ALB (gm/dl)	3.74.±0.25.	3.869 ±0.36	> 0.05	N.S.
III	TSP (gm/dl)	6.5 ± 0.67	6.669 ± 0.52	> 0.05	N.S.
	ALB (gm/dl)	3.76 ± 0.33	3.869 ± 0.36	> 0.05	N.S
IV	TSP (gm/dl)	6.86 ± 0.44	6.669 ± 0.52	> 0.05	N.S
	ALB (gm/dl)	3.94 ± 0.25	3.869 ± 0.36	> 0.05	N.S

Seven of these 30 patients 2 from group (I), 2 from group (II) and 3 from group (III) showed abnormally elevated levels of serum enzymes GOT, GPT, ALP, GGT, and TSB Table (4).
With respect to the remaining 23 patients with ALL under MT who were classified within 4 groups according to the dural of MT, 10 of them (5 patients within group (I) and 5 patients within group (II) have. insignificantly elevated levels of GOT, GPT in serum (higher than the control subjects group as well as the normal reference range) TSB level for these 23 patients was within the normal reference range and insignificantly differ from that of the control subjects. In spite of the slight increase in serum levels of ALP & GGT enzymes for 23 patients compared to those of control subjects, their activities were within normal references range Table (5)

Table 4 . Liver function tests for 7 patients with ALL under maintenance therapy with 6–mercaptopurine and methotrexate combination

Patient s names	Age (Years)	Duration of MT (months)	GOT U/L	GPT U/L	T.S.B mg/dl	GGT U/L	ALP K.K.U/dl
M.M	4	5	23	57	1.6	40	23
M.A.B	9	5	19	35	1.5	42	28
H.S	12	10.8	100	75	1.3	38	22
G.M	6	15.4	230	100	3.3	78.9	40
M.A	5.3	22	113	150	1.4	45.5	23
S.J	11.4	22.8	100	110	2.5	69.4	29
S.H	9	27.6	190	130	1.8	63	26
P value			< 0.001	<0.001	<0.01	<0.01	<0.05
significance			H..S	H..S	S	S	S

H.S = High Significant S = Significant

Table 5 . Liver function tests measured in the sera of 23 patients with ALL under MT.

Patients under MT	Age (Years) M±SD	Duration of MT (months) M±SD	GOT (U/L) M±SD	GPT (U/L) M±SD	ALP (K.K.U/dl) M±SD	GGT (U/L) M±SD	T.S.B (mg/dl) M±SD
5 patients within group I (under MT for up to 6 months)	8.2 ± 0.44	5.32 ± 0.46	18.8 ± 1.92	25.6 ± 8.91	17.2 ± 3.96	21.22 ± 4.27	0.56 ± 0.134
5 patients within group II (under MT for 10-18 months)	8.56 ± 3.9	14.1 ± 2.5	15.5 ± 6.04	19.75 ± 9.58	17.75 ± 3.05	23.75 ± 7.64	0.587 ± 0.18
5 patients within group III (under MT for 20-28 months)	9.24 ± 3.5	23.96 ± 1.26	10 ± 0.71	12.6 ± 1.82	15.2 ± 4.21	19.28 ± 6.71	0.58 ± 0.24
5 patients within group IV (under MT for 30-36 months)	9.36 ± 2.5	33.54 ± 2.84	10.6 ± 0.89	12.2 ± 0.84	16.2 ± 3.89	22.2 ± 8.34	0.62 ± 0.26

DISCUSSION:

ALL as a disease and before starting with chemotherapy is not associated with hepatotoxicity or liver cells damage. intrinsic hepatotoxicity", they induce structural changes of the hepatocyte by competitive inhibition of essential metabolites for the hepatocytes so these changes could be due to, the cytotoxic effect of these two agents[(4)].

The severity of MTX–induced liver damage could be related to both duration of therapy and the mode of administration, the prevalence of fibrosis and cirrhosis appeared significantly greater in subjects given frequent small doses of MTX than those given intermittent large doses[5].
The long–term use of 6MP in treatment of childhood ALL is associated with hepatotoxicity features of intrahepatic cholestasis and of parenchymal cell necrosis, either of which being predominate[6].
The pathological changes in liver diseases fall into two main groups; the liver cell damage (with or without demonstrable liver dysfunction), and the biliary tract involvement[7]. So the results of liver function tests may be characteristic of the under lying pathological process. High serum GOT, GPT levels, which are soluble cytoplasmic and mitochondrial enzymes in the hepatocyte, indicate liver cell damage (destruction)[7]. High serum ALP, GGT levels, which are hepatocyte membrane associated enzymes, indicate cholestatic lesion. Jaundice may or may not be present with any of these processes[7].
Serum total protein, albumin levels for the selected 30 patients with ALL under MT with MTX and 6MP combination were normal compared to those of the healthy control subjects, this is mainly due to the ability of the liver to increase protein, albumin biosynthesis during diseases associated with protein loss, or in presence of liver cell damage or injury induced by cytotoxic drugs, until the parenchymal damage or loss is sever (with the loss of 95% or so of function)[8, 9].
Seven of 30 patients with ALL under MT have both hepatocellular destruction (mainly induced by MTX) and intrahepatic cholestasis (mainly induced by 6MP) as they showed abnormally elevated levels of serum GOT, GPT, ALP, GGT enzymes and total serum bilirubin level.
Jaundice in these patients was due to obstruction of biliary canaliculus as a result of intrahepatic cholestasis. The liver injury associated with jaundice has been reported in 6–40% of ALL patients treated with 6MP[5].
For the remaining 23 patients with ALL within the four groups according to the duration of MT they received, it was found that serum total bilirubin.levels. were normal compared to those of the control subjects and were within the normal reference range for this parameter. Although serum ALP, GGT levels of these 23 patients within the four groups were somewhat higher than those of the control subjects, but they were within the normal reference range for each one of these two enzymes in serum. Serum GOT, GPT levels were abnormally higher than those of the control subjects in 13 of these 23 patients (5 patients within group(I) and 8 patients within group(II) this indicate the presence of liver cell destruction in these patients.
Pharmacogenetics is a Newfield of research in ALL, genetic differences between individuals affect their responses to drug therapy.
In treating childhood ALL, genetic variation in drug metabolism may have an important effect in treatment outcome[10].
6MP is inactivated by thiopurine. Methylene transferase in the liver. Mutation have been identified which result in reduced enzyme activity. ALL patients who are homozygous for the deficiency are at risk of. Life – threatening toxicity from their inability to metabolize normal doses of 6MP[11] Similarly patients with polymorphism in the gene coding for methelynetetrahydrofolate reductase, an important enzyme in folate metabolism have an increased risk of toxicity during MTX. Treatment[12]. It has been demonstrated that individualizing dosagesof6MP and MTX during MT by increasing them to the maximal tolerated level can improve survival provided that prolonged neutropeniais avoided[13]

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