

RESEARCH PAPER

## The Effect of Folate Status on Methotrexate Serum Level and Response in Children with Acute Lymphoblastic Leukemia

Dhuha Emad Omran<sup>1</sup>, Nabeel A.J. Ali<sup>2</sup> and Janan G. Hassan<sup>3</sup>

1. (B.Sc. Pharmacy/ master candidate), Department of Pharmacology, College of Medicine, University of Basrah, Basrah, Iraq.
2. Professor, Department of Pharmacology, College of Medicine, University of Basrah, Basrah, Iraq.
3. Department of Pediatrics, College of Medicine, University of Basrah, Basrah, Iraq.

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### Abstract

**Background:** Children in our area are more prone to folate deficiency due to many reasons including leukemia. The folate status of patients especially prior to therapy could influence MTX therapeutic effect or toxicity

**Aim:** To evaluate serum levels of folate in newly diagnosed children with ALL compared to healthy children, and to correlate the serum level and toxicity of MTX to folate status prior to and after therapy

**Patients and Methods:** A total of 34 children newly diagnosed with acute lymphoblastic leukemia, and 25 normal healthy children were included as a control group. Parameters measured include; serum folate, serum MTX, blood parameters, liver function tests, blood urea and serum creatinine that were measured before initiation of chemotherapy, during treatment and following completion.

**Result:** Newly diagnosed ALL children have low folate level at the time of diagnosis ( $5.26 \pm 3.82$  ng/ml, mean  $\pm$  SD) which is significantly lower than control group ( $11.16 \pm 5.20$  ng/ml, mean  $\pm$  SD) (P value < 0.0001). Leukemic children that have low folate is 20 with a serum level ( $3.12 \pm 1$  ng/ml, mean  $\pm$  SD), normal folate level is found in only 14 leukemic children and their level ( $8.83 \pm 4.14$  ng/ml, mean  $\pm$  SD). Low WBC and platelets count was detected following the first MTX dose which returns back to normal afterward. Folate serum level of all leukemic children at the end of all doses is significantly elevated ( $14.58 \pm 5.3$  ng/ml, mean  $\pm$  SD) (p value < 0.0001).

**Conclusion:** Serum folate level is low in a considerable number of newly diagnosed children with ALL before initiation of treatment protocol, this may to more hematological toxicity following the first MTX dose. It is recommended that serum folate should be measured and deficiency corrected prior to cytotoxic therapy to reduce toxicity.

**Keywords:** folate deficiency, methotrexate, acute lymphoblastic leukemia.

**\*Correspondence:** Dhuha Emad Omran, (B.Sc. Pharmacy/master candidate), Department of Pharmacology, College of Medicine, University of Basrah, Basrah, Iraq.

✉ Email Address: [dhuhaomran88@gmail.com](mailto:dhuhaomran88@gmail.com)

### Introduction

Acute Lymphoblastic Leukemia (ALL) is the most common malignancy of childhood, but fortunately, it also has the highest

cure rate. The incidence of the disease accounts for 30% of all childhood cancers and about 80% of all childhood leukemia.<sup>1</sup> When children with ALL treated with modern treatment protocols, about 85% of them are expected to be cured. Polymorphism in the gene encoding methylenetetrahydrofolate reductase (MTHFR) that result in alteration in folate metabolism along with low dietary folate intake, have been linked to a variety of diseases.<sup>2</sup> Standard treatment options for childhood ALL include different cytotoxic agents grouped into what is

called therapeutic phases or elements which include remission induction chemotherapy agents-vincristine, a corticosteroid, anthracyclines, and asparaginase. The consolidation phase includes cyclophosphamide, cytarabine, 6-mercaptopurine, and high-dose MTX. Most protocols also include an intensification phase, utilizing the same drugs, namely vincristine, corticosteroids, anthracyclines, and cytarabine, combined with another thiopurine, such as 6-thioguanine.<sup>3,4</sup> Side effects due to efficient therapy occur in 75% of childhood ALL patients, the adverse effects of treatment in pediatric ALL are outcomes of the poor specificity of drugs, the low therapeutic index of drugs and the high doses long-term regimens. Most common treatment complications include hypersensitivity reactions, hepatotoxicity, gastrointestinal and renal damage as well as myelosuppression<sup>5</sup> MTX acts by interfering with the metabolism of folic acid. When it pass to the cell, MTX is polyglutamated, binds dihydrofolate reductase (DHFR) with an affinity 1,000-fold greater than that of folate, and thus, it competitively inhibits conversion of dihydrofolate to tetrahydrofolate.<sup>6</sup> High dose MTXb therapy (HDMTX) used in leukemia can be severely toxic, which not only leads to morbidity and occasional mortality, but may also cause the interruption of treatment steps, potentially leading to inferior anticancer outcome. It is important to prevent or reduce the undesirable effects by a strict standardized supportive care.<sup>7</sup> Even when identical doses of HDMTX are given to patients, they vary significantly in their pharmacokinetics, patterns of toxicity and response to therapy. This diversity can, to some extent, be linked to sequence variations in genes involved in drug absorption, metabolism, excretion, cellular

transport, and/or effector targets pathways.<sup>8</sup> Folates which include folic acid and its derivatives have chemo protective effect and are considered micronutrients of great importance. They are water-soluble vitamins that act as co-factors in a variety of enzymatic reactions within the cell, leafy vegetables, eggs, legumes, bran and dry fruit are good sources of folates. Synthetic folates supplementations has greater bioavailability.<sup>9</sup> Deficiency of folates is linked with many health issues in both children and adults, like neural tube defects (NTDs) and malformations in the developing fetus and cardiovascular diseases, depression, Alzheimer's disease and megaloblastic anemia in adults.<sup>10</sup> The aim of the present study is to evaluate folate status in leukemic children prior to and during exposure to cytotoxic drug therapy, and to investigate the potential role in drug related toxicity and MTX serum level.

## Patients, Materials and Methods

### Patients

The study was conducted in Basrah Specialized Hospital for Children, during the period from June, 2018 until June 2019. Newly diagnosed children with acute lymphoblastic leukemia (ALL), admitted to the hospital for chemotherapy, that include MTX were included in the study. Their ages range from 15 months to 12 years. Patients with hemoglobinopathies (sickle cell disease and thalassemia) hemophilia and liver diseases were excluded from the study. Healthy children included as a control group aged between 16 months to 13 years, (Table-1) show the characteristics of all children included in the study. The diagnosis of ALL was confirmed in each patient by complete blood

picture, blood film and bone marrow test, conducted by the consultant hematologist. The characteristics of patients were determined by a special questionnaire format that was specially designed for this study, which includes patient's name, gender, date of birth, address and parent's phone number, date of diagnosis and date of initiation of treatment. A written informed consent was obtained from the child parents

which was written in Arabic language. Ethical approval of the study was obtained from both the Ethical Committee of Basrah College of Medicine and Basrah Health Directorate. Patients were followed-up by hematological and biochemical tests from the time of diagnosis with ALL, until they received the last intravenous dose of MTX.

**Table 1.** Characteristics of children included in the study

Group		Gender				Age (year) Mean ± SD	Weight (kg) Mean ± SD	Surface area (m <sup>2</sup> ) Mean ± SD
		Male		Female				
		No.	%	No.	%			
1	Newly diagnosed ALL patients	15	44.1	19	55.9	5.409 ± 2.99	19.96 ± 8.476	0.777 ± 0.231
2	Control group	12	48	13	52	7.05 ± 2.63	23.31 ± 7.92	-

#### ***Blood samples collection***

A blood sample of 2 ml volume was collected from each patient at each time via intravenous puncture. A gel containing tube was used for this purpose, the gel in the tube helps to separate serum from the rest of the blood components after centrifugation and stored until the time of analysis.

#### ***Measured parameters***

Serum MTX Serum folate, blood parameters including hemoglobin, WBC and platelets, total serum bilirubin, blood urea and serum creatinine.

#### ***Timing of folate and MTX measurement***

Patients with standard risk ALL received four high doses of MTX 2g/m<sup>2</sup> (methotrexate 500mg in 50ml vial, Ebewe, Austria) by intravenous infusion in 500 ml f glucose saline infusion according to their treatment protocol, The first blood sample was collected for each patient at the time of confirming the diagnosis to measure folate level at baseline. (Figure-1)

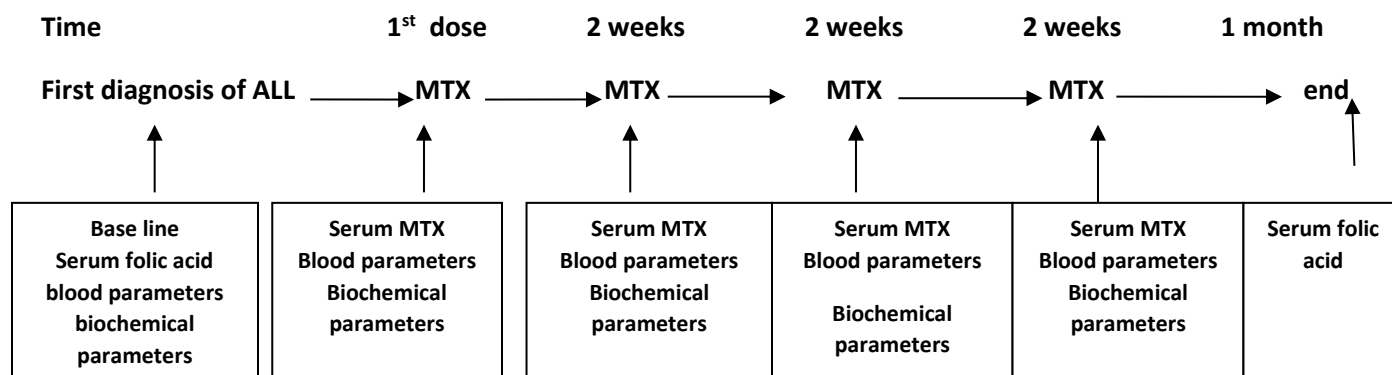


Fig 1. Schematic diagram illustrates time of blood sampling

The patients receive the rescue of folic acid (vial 10mg/5ml, Pfizer, USA) intravenously, given in a fixed dose of 15 mg/kg body weight at 42 hours after MTX this dose is also repeated in 48 and 56 hours after MTX infusion.

**Principle of the folate and MTX assay**

Both folate and MTX were measured by immunoassay method using Architect immunoassay analyzer that works by chemiluminescent microparticle. The overall specificity and sensitivity of this method is 99.6% and 99.7% respectively. These assays were carried out by “Al-Bayan, laboratories in Basrah.

**Statistical analysis**

Blood and biochemical parameters were measured by standard methods. Data were analyzed statistically by SPSS computer package version 24. Data are presented as mean ± SD

**Results**

**Folate serum level in ALL children and control group**

A statistically significant difference was found in folate serum levels between the newly diagnosed ALL children (5.26 ± 3.82 ng/ml,) and healthy children (11.16 ± 5.2 ng/ml, mean

± SD). Children with ALL were divided into two groups, low serum folate group and normal serum folate group. Of the 34 leukemic low folate level detected in 20 (58.9%), while normal folate level was found in 14 leukemic children as shown in (Table-2).

Table 2. Folate serum levels in the newly diagnosed all patients and control group

Group		No. of patients	Folate serum level (ng/ml) Mean ± SD	P value
1	Patients with folate level < 5 at time of diagnosis	20	3.12 ± 1	< 0.0001*
2	Patients with folate level ≥ 5 at time of diagnosis	14	8.833 ± 4.14	
3	Total ALL patients	34	5.263 ± 3.821	< 0.0001*
4	Normal healthy children	25	11.16 ± 5.2	

\*t test was used to measure p value

### Blood parameters of the patients at time of diagnosis

All the patients have low hemoglobin level with a mean of  $(8.1 \pm 2.68 \text{ g/dL})$ . The low folate group, Hb level was  $(7.6 \pm 2.2 \text{ g/dL})$ , while the normal folate group, Hb was  $(8.91 \pm 3.23 \text{ g/dL})$ . There was no difference between total WBC count in patient with low folate  $(53.57 \pm 57.66 \times 10^3/\mu\text{L})$  as compared to normal folate level  $(67.93 \pm 57.45 \times 10^3/\mu\text{L})$ . There was a significant correlation between serum folate level and Hb level ( $r= 0.23, n=32$ ). There were no statistically significant correlations between folate serum level and total WBC and platelets counts.

### Biochemical parameters of the patients

No statistical differences were detected between various biochemical parameters

### The effect of high dose MTX during the first dose

Patients with standard risk ALL received four high doses of MTX (2g/m<sup>2</sup>) according to their treatment protocol. The baseline serum folate level was correlated to the serum level of MTX. The mean serum MTX level was  $(4.97 \pm 3.6 \mu\text{g/ml})$  in patients with low folate level and this was comparable to patients with higher folate level  $(4.71 \pm 3.6 \mu\text{g/ml})$  (Table-3), a positive correlation ( $r =0.37, n=22$ ) was found between dose and serum MTX level. Both groups have low hemoglobin level, and patients with normal folate level tend to have higher WBC and platelets and it was statistically significant from those with low folate. There was a significant correlation between serum MTX and Hb level.

**Table 3.** Methotrexate serum level and blood parameters of the patients after the first dose.

No.	Group	No. of patients	MTX serum level ( $\mu\text{g/ml}$ ) Mean $\pm$ SD	Hb (g/dL) Mean $\pm$ SD	WBC ( $\times 10^3/\mu\text{L}$ ) Mean $\pm$ SD	Platelets ( $\times 10^3/\mu\text{L}$ ) Mean $\pm$ SD
1	Patients with folate level $< 5$ at time of diagnosis	15	$4.97 \pm 3.6$	$9.7 \pm 2.03$	$1.66 \pm 0.99$	$252.56 \pm 144.23$
2	Patients with folate level $\geq 5$ at time of diagnosis	7	$4.71 \pm 3.6$	$10.83 \pm 0.93$	$3.88 \pm 1.16$	$489 \pm 149.7$
3	Total	22	$4.884 \pm 3.52$	$10.04 \pm 1.81$	$2.35 \pm 1.467$	$326.46 \pm 180.71$
P value			0.8762**	0.1791**	0.0002**	0.0021**

\*\*t test was used to measure P value

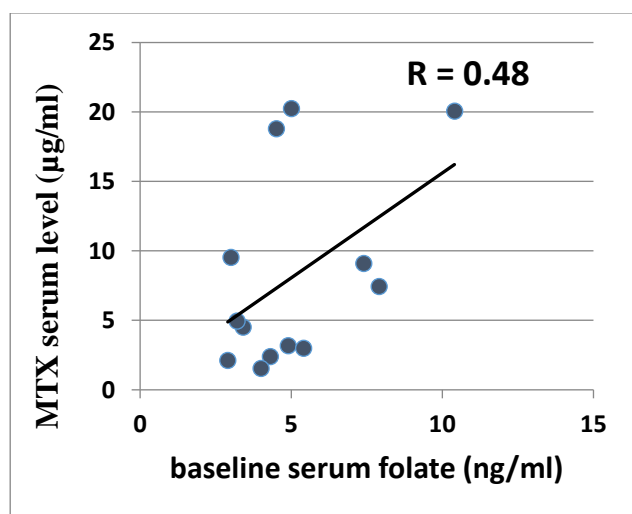
### The effect of high dose methotrexate during the second dose

The second MTX dose was administered after 2 weeks of the first one for patients with standard risk leukemia. The serum MTX level was higher in patients with normal folate level  $(11.75 \pm 7.78 \mu\text{g/ml}, \text{mean} \pm \text{SD})$  as compared with those of low folate level  $(5.87 \pm 5.8 \mu\text{g/ml}, \text{mean} \pm \text{SD})$  as shown in (Table-4.).

**Table 4.** Methotrexate serum level ( $\mu\text{g/ml}$ ) in different patients

Group	1 <sup>st</sup> dose MTX	2 <sup>nd</sup> dose MTX	3 <sup>rd</sup> dose MTX	4 <sup>th</sup> dose MTX
Patient with folate $< 5$	$4.9 \pm 3.6$	$5.87 \pm 5.8$	$7.09 \pm 5.4$	$6.28 \pm 5.3$
Patient with folate $> 5$	$4.71 \pm 3.6$	$11.75 \pm 7.8$	$6.6 \pm 4.6$	$6.29 \pm 4.3$
Total	$4.88 \pm 3.5$	$8.03 \pm 7.0$	$6.9 \pm 5$	$6.29 \pm 4.8$

Both groups have low Hb level and similar values of WBC and platelets. No statistically significant correlations were found between serum MTX and various blood parameters. A significant correlation was found between baseline serum folate and MTX serum level (Figure 2).



**Fig 2.** Correlation between baseline serum folate and MTX serum level

***The effect of high dose methotrexate during the third dose***

The third dose was administered at 2 weeks after the second one for patients with standard risk leukemia. Both groups have similar MTX level, in low folate group ( $7.09 \pm 5.36 \mu\text{g/ml}$ , mean  $\pm$  SD) and in normal folate group ( $6.6 \pm 4.55 \mu\text{g/ml}$ , mean  $\pm$  SD). The different blood parameters are similar in both groups.

***The effect of high dose methotrexate during the fourth dose***

The fourth dose was given after 2 weeks in patients with standard risk leukemia. MTX serum level in low folate group ( $6.28 \pm 5.32 \mu\text{g/ml}$ , mean  $\pm$  SD) which is very close to that

in normal folate group ( $6.29 \pm 4.29 \mu\text{g/ml}$ , mean  $\pm$  SD). There were no significant differences between the various blood parameters with slight improvement in Hb level from the previous reading.

***End line folate measurements of the patients***

After finishing all doses of MTX, another folate measurement was done. There was a statistically significant difference between baseline ( $5.263 \pm 3.821 \text{ ng/ml}$ ) and end line folate measurement ( $14.58 \pm 5.3 \text{ ng/ml}$ ) in the newly diagnosed ALL children. The low folate group started with folate serum level ( $3.12 \pm 1 \text{ ng/ml}$ ) and end with ( $13.73 \pm 5.53 \text{ ng/ml}$ ), while the normal folate group started with folate serum level ( $8.83 \pm 4.14 \text{ ng/ml}$ , mean  $\pm$  SD) and end with ( $17.7 \pm 3.3 \text{ ng/ml}$ ). (Table-5)

**Table 5.** End line folate serum level and blood parameters of the patients

	Group	No. of patients	Folate (ng/ml) Mean $\pm$ SD	Hb (g/dL) Mean $\pm$ SD	WBC ( $\times 10^3/\mu\text{L}$ ) Mean $\pm$ SD	Platelets ( $\times 10^3/\mu\text{L}$ ) Mean $\pm$ SD
1	Patients with folate level < 5 at time of diagnosis	11	13.73 $\pm$ 5.53	10.71 $\pm$ 2.2	3.75 $\pm$ 2.4	277.27 $\pm$ 165.2
2	Patients with folate level $\geq$ 5 at time of diagnosis	3	17.7 $\pm$ 3.3	9.6 $\pm$ 0.55	3.08 $\pm$ 0.92	303.1 $\pm$ 120.2
3	Total	14	14.58 $\pm$ 5.3	10.4 $\pm$ 1.9	3.55 $\pm$ 2	285 $\pm$ 146.86

**Discussion**

***Folate serum level before the induction phase***

Deficiency of folate in leukemic patients is interesting, it was explained by increase demand for folates by the leukemic cells, also it was thought to be the result of impaired intestinal absorption.<sup>11</sup> In the present study, leukemic patient has significantly low folate level at the time of diagnosis associated with low Hb. This result is comparable with a study by Tandon and his colleagues in a tertiary care

Teaching Hospital in Northern India, in which folate level assessment was done before the induction phase and found folate deficiency in 41.3% of children with ALL with a mean of (8.5 ng/ml) and the a range of (1.28 ng/ml -20 ng/ml).<sup>12</sup> In another study by Jaroslav Sterba and his colleagues, folate serum level was measured to assess the effect of pretreatment folate level on pharmacodynamics of high dose MTX in 32 children that are newly diagnosed with ALL. Results showed low folate level with a median value of (3 ng/mL) and a range of (1.58 ng/mL - 16.8 ng/mL). In a case-control study carried out in India it was found that folate levels were significantly lower among cases as compared to control group (8.56 ± 4.35 ng/ml ) vs. (14.04 ± 2.62 ng/ml).<sup>13</sup> Factors that may contribute to the low folate include infection, malnutrition and defective absorption, which are common in the developing countries.<sup>14</sup>

#### ***Folate and methotrexate***

The serum folate levels were similar in both the folate normal and the folate deficient groups, apart from the second dose during which there was significant differences between the two groups. This result is probably due to a possible interaction, as folic acid can inhibit the enzyme aldehyde oxidase that metabolize MTX.<sup>15</sup> Measurement of methotrexate serum levels during high dose therapy in leukemic children is recommended specially during the use of folinic cid rescue. We found a positive correlation between the dose of MTX and its serum level, therefore; therapeutic monitoring of MTX serum level is important during high dose therapy in ALL patients to avoid toxicity. It was found that MTX serum level monitoring may be a limited predictor of toxicity.<sup>16</sup>, it was also linked to improving the outcome in

children with ALL.<sup>17</sup> In other uses of MTX (Rheumatoid Arthritis.<sup>18</sup> Psoriasis (Arabelovic S et al, 2007) and Ectopic Pregnancy<sup>19</sup>, it seems that using folinic acid rescue doses or having high pretreatment folate level may decrease the efficacy of MTX in these indications and lead to failure of treatment. This suggests a therapeutic window for folinic acid effect on methotrexate. MTX can interact with folate in either way, it can reduce the folate level by accelerating its catabolism which is extremely slow in absence of MTX.<sup>20</sup> Folic acid can reduce MTX level by inhibiting the enzyme aldehyde oxidase, the main catabolic pathway of MTX which convert it to 7-OH-MTX.<sup>21</sup> At the present study, folate serum level was measured after finishing the consolidation phase (at the patient's next visit to the hospital) which includes a course of four doses of MTX. Folate serum level at this point is elevated (Mean ± SD 14.58 ± 5.3 ng/ml) vs. (Mean ± SD 5.26 ± 3.82 ng/ml) at baseline due probably to clinical response and leucovorin rescue therapy. A statistically significant difference is found between baseline folate serum level and after finishing the consolidation phase, there is a significant increase in folate serum level after four HDMTX, which could be due to folinic acid dose or due to clinical improvement. There are many explanations for folate increment after HDMTX like blood transfusion, multivitamins intake, reflected recovery from the leukemia, improved dietary intake and the effect of folinic acid rescue doses after each HDMTX. The increment in folate serum level after end of consolidation phase can be beneficial as side effects associated with HDMTX are decreased, however; this increment may have drawback at least theoretically as it can reduce MTX

efficacy or cause cancer relapse.<sup>22</sup> In a study by Natanja Oosterom et al<sup>23</sup> in children with ALL, they found a significant increment of folate serum level from (7.2 ng/ml) to (18.8 ng/ml) following four HDMTX. Another study also agree with the present study in that folate serum level is increased after HDMTX that found serum and erythrocyte folate pretreatment concentrations increased significantly with increasing number of HD MTX courses in patients with osteosarcoma.<sup>24</sup> It is apparent that there is a critical balance in the body folate status and therapeutic outcome of MTX, this requires careful supplement with folic acid to avoid toxicity and ensure good therapeutic outcome. A single folate measurement in serum may not reflect the real value, instead; the mean of repeated values would be more accurate. Limited number of patients due to the short study period is one of the limitations of the current study.

## Conclusions

Serum folate is low in a considerable number of the newly diagnosed children with ALL before the initiation of treatment protocol. The low folate level is associated with more hematological toxicity of MTX, especially during the initiation of therapy. A positive correlation between the dose of MTX and its serum level, serum level measurement might be useful in dose adjustment

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## تأثير حالة الفولات على مستوى مصل الميثوتريكسيت والاستجابة عند الاطفال المصابين بسرطان الدم الليمفاوي

**الخلفية:** يعتقد ان الاطفال فى منطقتنا معرضين للإصابة بنقص مادة الفوليت نتيجة لعدة عوامل منها اللوكيميا. ان حالة الفوليت فى الجسم خاصة قبل اعطاء العلاج للمرضى ممكن ان تؤثر على الاستجابة لعقار الميثوتريكسيت وسميته.

**المرضى والطريقة:** الاطفال المصابين بداء اللوكيميا الحاد والمشخصين حديثا (العدد=34) والاطفال الاصحاء (العدد=25) قد اشتركوا بهذه الدراسة. يتم اخذ عينات من دم المرضى لأجراء الفحوصات التالية: مستوى عقار الميثوتريكسيت فى مصل الدم, مستوى مادة الفوليت فى مصل الدم, معالم الدم ويشمل نسبة الهيموغلوبين وتركيزه فى الخلايا وعدد كريات الدم البيضاء والصفائح, وظائف الكبد ويشمل البليروبين ونسبة اليوريا والكريتينين. وقد تم قياسها قبل واثناء وبعد اعطاء العلاج للمرضى.

**النتائج:** الاطفال المشخصين حديثا باللوكيميا الحادة ينخفض لديهم مستوى الفوليت فى مصل الدم عند وقت التشخيص بالمرض (  $3,82 \pm 5,26$  نانوغرام/ مل ) وهو اقل من مستوى نفس المادة لدى الاطفال الاصحاء ( $p < 0,0001$ ) (11,16)

ان عدد الاطفال المصابين باللوكيميا الحادة ولديهم مستوى فوليت منخفض هو 20 ومعدل الفوليت لديهم (3,21  $\pm$  1 نانوغرام/ مل ) بينما كانت مستويات نفس المادة طبيعة لدى 14 طفل فقط (  $4,14 \pm 8,83$  نانوغرام/ مل). وجدت مستويات منخفضة من كريات الدم البيضاء وصفائح الدم بعد انتهاء الجرعة الاولى من عقار الميثوتريكسيت ثم ارتفعت بعد الجرعة التى تليها. ارتفع مستوى الفوليت لدى جميع الاطفال المرضى بعد انتهاء العلاج (  $5,3 \pm 14,58$  نانوغرام/ مل) ( $p < 0,0001$ ) مقارنة مع القراءة الأولية.

**الاستنتاج:** ان مادة الفوليت فى مصل الدم واطنة بعدد غير قليل من الاطفال المشخصين حديثا بلوكيميا الدم الحاد وهو اقل بكثير من مستوى نفس المادة لدى الاطفال الاصحاء وان انخفاض هذه المادة مرتبط مع سمية اكبر لعقار الميثوتريكسيت تظهر على معالم الدم. من الضروري قياس مادة الفوليت منذ البداية مع تعديل اى نقص فيها قبل البدء بالعلاج لتقليل سميته.

**الكلمات المفتاحية:** نقص الفوليت، الميثوتريكسيت، داء اللوكيميا الحاد