Effect of ursodeoxycholic acid and vitamin E in the prevention of liver injury from methotrexate in pediatric leukemia

Mohammadreza Bordbar[1], Nader Shakibazad[1], Mohammadreza Fattahi[2], Sezaneh Haghpanah[1], Naser Honar[3]

[1]Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Gastroenterology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of Gastroenterology, Shiraz University of Medical Sciences, Shiraz, Iran

Cite this article as: Bordbar M, Shakibazad N, Fattahi M, Haghpanah S, Honar N. Effect of ursodeoxycholic acid and vitamin E in the prevention of liver injury from methotrexate in pediatric leukemia. Turk J Gastroenterol 2018; 29: 203-9.

ABSTRACT

Background/Aims: Ursodeoxycholic acid (UDCA) and antioxidants such as vitamin E are considered to have a protective role in preventing chemotherapy-induced liver damage. The aim of this study was to assess the efficacy of these agents for hepatoprotection in pediatric patients with B-cell acute lymphoblastic leukemia (ALL), who were treated with methotrexate in their maintenance phase of treatment.

Materials and Methods: Eighty children with B-cell ALL were randomly divided into four groups. Group 1 was administered oral vitamin E (400 mg/day); group 2 was administered oral UDCA (15 mg/kg/day); group 3 was administered a combination of the two drugs; and group 4 served as a control group and was administered no drug except their chemotherapy drugs. Complete blood count, liver function test, liver ultrasonography, and liver fibroscan were requested, and the results were compared.

Results: Group 1 showed a slight increase in total bilirubin levels compared to baseline levels during the study (P=0.036). Group 2 showed a decline in aspartate aminotransferase and alanine aminotransferase levels during the study and at 6 months after discontinuing the drug; however, these differences were not statistically significant (P=0.051 and 0.083, respectively). None of the patients showed the evidence of significant fibrosis on liver fibroscan. Eight patients showed some evidence of mild-to-moderate fibrosis (F1, F2), but the results were not different between the groups as well as between pre- and post-study periods in each group.

Conclusion: Low-dose methotrexate does not cause significant liver fibrosis in pediatric leukemia. UDCA and vitamin E have minimal roles in hepatoprotection among pediatric patients with ALL.

Keywords: Antioxidants, ursodeoxycholic acid, vitamin E, methotrexate, hepatotoxicity

INTRODUCTION

Metabolic detoxification is one of the liver functions, which has an important role in preventing the metabolite toxicity of drugs. While this function predisposes liver to damage, some medications are identified that induce liver damage more commonly than others (1,2). The liver damage induced by chemotherapy agents can manifest in a number of forms varying from an asymptomatic patient with mild elevation in transaminases on laboratory study to a severely ill patient presenting with a condition such as acute viral hepatitis (3). Methotrexate (MTX) is an anti-metabolite agent that inhibits folate metabolism and can be used for different indications. MTX-induced liver damage may occur in more than 10% of patients receiving MTX. The reduction of folate supplies causes inappropriate (DNA) replication inside hepatocytes, leading to the accumulation of toxins in the liver and raising aminotransferase levels. Therefore, hepatotoxicity remains a major concern among pediatric patients with acute lymphoblastic leukemia (ALL) in their maintenance phase of treatment, which includes the weekly ingestion of low-dose oral MTX (1,3,4).

Several agents, including ursodeoxycholic acid (UDCA), omega-3 fatty acids, black seed oil, virgin coconut oil, and vitamin E, are used to prevent chemotherapy-induced liver damage (5-9).

ORCID IDs of the authors: M.B. 0000-0002-8071-6425; N.S. 0000-0002-5124-6380; M.F. 0000-0000-6160-0403; S.H. 0000-0002-8666-2106; N.H. 0000-0002-7993-2681.

Address for Correspondence: **Naser Honar** E-mail: **honarn@sums.ac.ir**
Received: **August 19, 2017** Accepted: **November 21, 2017**

DOI: **10.5152/tjg.2018.17521**

The objective of this study was to investigate the clinical hepatoprotective effect and safety of UDCA and/or vitamin E in pediatric patients with ALL, who were in the maintenance phase of treatment. In addition, this study assesses whether the combination of the two drugs had an additive effect compared with the single use of each drug for preventing the fibrosis and the elevation of enzymes in the liver.

MATERIALS AND METHODS

In this open-label, randomized clinical trial, we included 80 children (age range, 2-18 years) with consecutive precursor B-cell ALL, who were in the maintenance phase of chemotherapy for at least 6 months. All patients presented with the same type of ALL. The regularly used chemotherapy agents included monthly vincristine injection, oral mercaptopurine 50 mg/m^2 every night, oral prednisolone 40 mg/m^2 for 5 consecutive days each month, and oral methotrexate 15 mg/m^2 every week. Therefore, all patients received the same treatment at the same time. Patients with viral, autoimmune, or a metabolic evidence of liver disease were excluded from the study. Patients were recruited over 1 month from a pediatric oncology outpatient clinic. The Ethics Committee of the University approved the study, and the study was registered with ID: IRCT2013120515666N1.

All the patients or their guardians signed the written consent form. The patients were randomized using block randomization method into four groups, with each group containing 20 patients.

Group 1 was treated with vitamin E (E-Zavit, 400 milligrams Capsule, Zahravi Pharmaceutical Co.) daily along with their routine chemotherapy drugs. Group 2 was treated with UDCA (Ursodiol, 300 milligrams Capsule, Alborz Darou Pharmaceutical Co.) at a dosage of 15 mg/kg/day along with their routine chemotherapy drugs. Group 3 was treated with a combination of the two drugs, and the last group was not treated with any drug except their routine chemotherapy agents, which was considered as the control group. The trial continued for a period of 6 months until the supplements (vitamin E and UDCA) in the first three groups were discontinued. In all included patients, baseline liver ultrasonography was performed by an expert radiologist. Complete blood count (CBC) and liver function tests (LFT) were conducted from all patients before participating in the study, at every month until the end of the study, and continually for another 6 months after the discontinuation of the drugs. Mean values of parameters were measured during the 6 months of the study (mean 1) and also during 6 months after study termination (mean 2). The results were compared within and between the groups.

Two patients (one in group 2 and one in group 3) failed to complete the study due to poor compliance with drug consumption and were excluded from the study. Therefore, the study was completed with 78 patients.

We used some formulas as indirect serum markers of liver fibrosis to assess the extent of liver injury between the groups. These included aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio, AST-to-platelet-ratio Index (APRI), and fibrosis-4 (FIB-4) score. APRI is calculated as follows: (AST/upper limit of normal range)/platelet count (10^9/L)×100. Scores <0.5 indicate no fibrosis, whereas scores >1.5 indicate significant fibrosis. FIB-4 score is calculated as follows: [age (year)×AST (U/L)]/[platelet (10^9/L)×ALT (U/L)$^{1/2}$]. Scores >1.45 indicate significant fibrosis (10).

At the end of the study, liver stiffness was measured in all patients by transient elastography (FibroScan) (Echosens, Paris, France). Liver stiffness was evaluated while the patient was lying on dorsal decubitus position, with the arms in maximal abduction. The measurements were taken in the right and left intercostal spaces (11). Liver stiffness was expressed in kilopascals (kPa) and was computed for each subject as the median of 10 validated measurements in accordance with the manufacturer's instructions.

Measurements with an interquartile range of <30% of the median value and a success rate of >60% were considered reliable. Two-dimensional shear wave elastography (SWE) studies were performed using the Aixplorer ultrasound system (SuperSonic Imagine SA, Aix-en-Provence, France) with a convex broadband probe (SC6-1, SuperSonic Imagine). This technique has an advantage that the probe can be installed on ultrasound machines. At the time of SWE examination, the patients were asked to hold their breath for 3 to 4 s. Liver stiffness was recorded in the right lobe, while the patient was lying on dorsal decubitus position, in accordance with the protocol used for FibroScan. An SWE box was placed 1.5 to 2 cm away from the Glisson capsule and on liver parenchyma to avoid measurements of large vessels. For quantitative

measurements, a round region of interest was placed inside the SWE box, and minimum and maximum values of stiffness expressed in kPa were recorded. Four measurements were made, and the median value was recorded. Metavir fibrosis score, which is graded on a 5-point scale from 0 to 4, was used to delineate the degree of fibrosis.

Statistical analysis

Data were analyzed using the SPSS (IBM Inc.; SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA). Descriptive data were presented as mean, standard deviation, and percentages. Chi-square test was used for the comparison of qualitative variables among different groups. McNemar's test was used for the comparison of qualitative variables in each group on different occasions. Analysis of variance (ANOVA) and Kruskal-Wallis H tests were used to compare quantitative variables among different groups. P-value <0.05 was considered statistically significant.

RESULTS

Seventy-eight patients (mean age, 7.2±4.2 years) completed the trial, and 66.7% of them were males. Demographic characteristics of the study population and the results of their liver ultrasonography are summarized in Table1. In total, 11 patients exhibited some minor abnormality in their liver ultrasonography before entering the study, including mild hepatomegaly and slightly increased parenchymal echogenicity, which were not significantly different among the four groups (p=0.77). However, follow-up ultrasonography at the end of the study (6 months later) revealed that 7 of 11 abnormalities were resolved, but their differences were not statistically significant (p>0.05). Group 1 exhibited the most resolved abnormalities (three patients), followed by group 4 (two resolved patients). Groups 2 and 3each had one case with resolved liver abnormality screened by ultrasonography.

During the study, 21 patients had to temporarily hold the use of MTX due to liver enzymes elevated to more than four-fold the upper normal limit. Most of these patients belonged to group 2 (8 patients), followed by group 3 (7 patients), which could be due to the problem of random selection, groups 1 and 4 each had three patients who were required to temporarily hold their drugs, and the results showed borderline statistical significance (p=0.05). The cumulative dose of MTX during the study period (6 months) was 360 mg/m^2.

Baseline laboratory data were comparable among the four groups of patients. Evaluated laboratory parameters included white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hb), platelet count (Plt), total and direct bilirubin, total protein, albumin, AST, ALT, and alkaline phosphatase (p>0.05).

A pairwise comparison was performed for the laboratory data in each group at three occasions: baseline and during the study (mean 1); at baseline and at 6 months after the completion of the study (mean 2); and between mean 1 and 2. Regarding CBC, the difference between mean 1 and 2 of total WBC and ANC in group 1 was statistically significant (3016±807/mm^3 vs. 3478±891/mm^3 and 1562±650/mm^3 vs. 2067±977/mm^3, respectively) (p=0.015 and 0.034, respectively). In other words, the mean WBC count and ANC during the 6-month period after vitamin E withdrawal were significantly higher than those during the period when vitamin E was being used. Moreover, the mean platelet count during the study (mean 1) was significantly different compared with baseline platelet count in the control group (234±106×109 vs. 274±71×109, respectively) (p=0.036). When the results were compared between the groups, none of the parameters showed a significant difference.

Considering LFT, the only parameter that significantly changed was the difference between mean 1 and baseline total bilirubin in group 1, which increased in the study period (mean 1) compared to the baseline values (0.767±0.265 vs. 0.629±0.177 mg/dL, respectively) (p=0.036). In patients receiving UDCA (group 2), AST showed a decreasing trend from baseline to mean 1 and 2 with marginal statistical significance. Mean AST changed from 58.88±43.84 mg/dL at baseline to 45.66±19.27 mg/dL during the 6-month study while using UDCA and 36.97±11.29 mg/dL at the end of 6-month follow-up after study termination (p=0.051). Similarly, mean ALT changed in this group from 123.23±158.15 mg/dL before entering the study to 46.78±29.98 mg/dL at 6 months after the discontinuation of UDCA. However, this trend did not reach statistical significance (p=0.083).

When indirect serum markers of hepatic fibrosis (AST/ALT ratio, APRI, and FIB-4 score) were compared in each group before and after the trial, none of the variables were significantly different. In addition, the comparison of the results between the groups was not statistically significant (p>0.05).

Table 1. Comparison of demographic and clinical characteristics among four groups of patients

Groups / Parameters	Groups 1 N=20	Groups 2 N=19	Groups 3 N=19	Groups 4 N=20	p
Age (year) Mean±SD	6.4±4.2	8.8±5.3	7.9±4.4	6.0±2.5	0.147
Sex (male) Number (%)	12 (60%)	12 (63.2%)	15 (78.9%)	13 (65%)	0.611
Duration of disease (month) Mean±SD	34±11	33±12	36±13	37±15	0.853
Duration of maintenance therapy (month) Mean±SD	27±12	26±13	29 ± 13	30±15	0.762
Mean of baseline ALT (U/L) Mean±SD	52.5±66.2	94.6±109	76.3±64.7	49.3±59.6	0.273
Mean of ALT during treatment (U/L) Mean±SD	59.2±108.1	48.1±28.8	68.5±70.4	47±31.2	0.92
Mean of baseline AST (U/L) Mean±SD	52.1±51.6	58.8±43.8	55.6±35.3	38.3±16.9	0.423
Mean of AST during treatment (U/L) Mean±SD	28.8±11.2	35.9±19	41.8±28.8	36±17.3	0.442
Abnormal ultrasonography (baseline) Number (%)	3 (15%)	2 (10.5%)	4 (21.1%)	2 (10%)	-
Abnormal ultrasonography (after 6 months) Before					
Number (%)	0	1 (5.3%)	3 (15.8%)	0	-
p	-	>0.999*	>0.999*	-	
Number of methotrexate withdrawal Mean±SD	0.2±0.5	0.7±1.0	0.7±1.1	0.2±0.4	0.05

ALT: alanine aminotransferase; AST: aspartate aminotransferase; SD: standard deviation
McNemar's test was used to compare ultrasonography findings before and after treatment; the comparison was impossible in groups 1 and 4 due to zero number of abnormal ultrasonography findings in the second examination

Finally, we performed liver fibroscan at the end of the study when the drugs were discontinued. The details of the measured parameters are summarized in Table 2. The patients receiving vitamin E as their supplements (group 1) showed no evidence of fibrosis in their fibroscan, and all of the scores with no fibrosis was classified as F0. Two patients receiving UDCA (group 2) showed mild-to-moderate fibrosis on fibroscan, which was classified as F1 (portal fibrosis without septa) and F2 (portal fibrosis with few septa). Three patients in group 3 showed some degree of mild fibrosis (two patients categorized as F1 and one as F2). Those who were followed-up as the control group (group 4) showed results similar to those of group 3 (i.e., three patients classified as F1). None of the studied patients showed the evidence of severe fibrosis on fibroscan (F3 or F4). Statistical analysis revealed no significant difference between groups in terms of fibrosis and steatosis scores measured by fibroscan (p>0.05).

It is worth mentioning that no complication associated with vitamin E and UDCA use was observed in our study patients.

DISCUSSION

The present study is the first randomized clinical trial of pediatric patients with ALL comparing the efficacy of vitamin E as an antioxidant and UDCA or a combination

Table 2. Comparison of demographic and clinical characteristics among four groups of patients

Groups / Parameters	Group 1 N=20	Group 2 N=19	Group 3 N=19	Group 4 N=20	p
Patient score	4.06±0.78	4.38±1.47	4.82±1.40	4.43±0.93	0.281
Success rate	98.10±3.78	94.66±6.97	142.88±216.68	95.00±9.64	0.457
IQR† Metavir	17.63±6.27	16.72±6.60	15.05±6.13	15.10±6.23	0.532
CAP‡ score	188.0±44.90	194.70±82.26	131.50±38.12	203.50±30.50	0.160
Steatosis percentage	5.60±3.20	23.75±34.64	4.0±1.41	7.33±2.65	0.567

All data are presented as mean±standard deviation
†IQR: interquartile range; ‡ CAP: controlled attenuation parameter
Cap score and steatosis percentage were compared by Kruskal-Wallis test among four groups due to missing data; in other variables, analysis of variance (ANOVA) was performed

therapy for protection against chemotherapy-induced liver damage, particularly that induced by MTX.

Our results did not support the beneficial role of vitamin E and UDCA in preventing the hepatotoxicity of low-dose MTX in patients with ALL.

Ursodeoxycholic acid may protect the liver by several mechanisms. UDCA has a role in the stabilization of plasma and mitochondrial hepatocytes membranes because it can protect hepatocytes from toxin, drugs, and other agents damaging liver. The membrane permeabilization of mitochondria in response to oxidative stress or DNA damage can induce apoptosis. Therefore, UDCA has an anti-apoptotic effect on hepatocytes by stabilizing the hepatocyte membrane (8,12).

Lapenna et al. (13) have confirmed that UDCA has an antioxidant and antilipoperoxidative effect as well as causes the immunomodulation and inhibition of phospholipid peroxidation (8). Moreover, UDCA has been widely used as a choleretic agent in the treatment of biliary cirrhosis and hyperbilirubinemia, particular unconjugated hyperbilirubinemia (14-16).

Vitamin E is considered to reduce the level of free radicals generated from oxidation. It also increases the level of glutathione peroxidase and decreases the level of malondialdehyde in patients with ALL who received it as a supplement. Thus, vitamin E can be used as an antioxidant in patients receiving chemotherapy (7,13).

Some patients with cancer use antioxidants as nutritional supplements during chemotherapy to alleviate treatment toxicities and to increase long-term survival. Nevertheless, little is known regarding the effectiveness and safety of antioxidant use during chemotherapy (17). There is a major concern regarding the possible interaction of antioxidants with chemotherapy agents (18).

As mentioned in the Results section, serum AST and ALT levels declined in the groups 1 and 2 compared to the control group, but their difference was not statically significant. In addition, indirect serum markers of hepatic fibrosis did not improve with the use of UDCA and vitamin E. Thus, the concurrent use of UDCA and vitamin E with chemotherapy does not appear to have beneficial effects.

However, none of the studied patients showed the evidence of severe persistent hepatic fibrosis with the use of oral low-dose MTX every week in ultrasonography and fibroscan as well as in the examination of serologic markers. Thus, the transient elevation of liver transaminases during chemotherapy may be safely managed with dose reduction or postponing the chemotherapy cycles.

Some studies have raised a concern regarding the safety of antioxidants along with chemotherapy in patients with cancer because antioxidants may protect both normal and malignant cell from free radicals (19).

Methotrexate can induce apoptosis in hepatocytes by increasing tumor protein 53 (TP53) levels. MTX also causes oxidative tissue injury in the liver by increasing reactive oxygen metabolites levels and lipid peroxidation. These mechanisms could be inhibited by using UDCA (8,9). However, our results did not support hepatoprotective effects of UDCA in pediatric patients with ALL receiv-

ing MTX in the maintenance phase of treatment. Larger multicenter studies with longer follow-ups are required to elucidate this controversial issue. Furthermore, we administered UDCA at the dosage of 15 mg/kg/day, and higher doses may prove to be more effective.

Vitamin E or UDCA was not effective in the prevention of mild-to-moderate liver fibrosis in Group 1, 2 or 3 compared to the control group. In addition to the small sample size that precludes a general conclusion, it should be noted that fibroscan has limited accuracy in detecting early stages of liver fibrosis. Although none of our patients showed signs of severe fibrosis, it is possible that a greater proportion of our patients in fact presented with mild fibrosis that was not detected by fibroscan.

This study has some limitations. First, the small sample size in each group made it difficult to draw a more reliable conclusion in some aspects. For instance, the beneficial role of UDCA in reducing elevated liver enzymes was equivocal with borderline statistical significance. The results might change if a larger population was studied. Moreover, we did not perform liver biopsy as the gold standard test to confirm liver cirrhosis (10). Because liver biopsy is an invasive procedure, we decided to substitute this with less invasive tests, such as fibroscan. Now, the use of non-invasive procedures could be recommended as screening tools that may help physicians restrict the patients' population to those who require a definitive testing of liver fibrosis, such as a liver biopsy.

While we failed to prove the true benefits of supplements, such as vitamin E or UDCA, in addition to chemotherapy, further studies should be conducted to evaluate the long-term benefit of these adjuvant therapies during the maintenance chemotherapy for the evaluation of survival outcomes, including disease-free and overall survival. Furthermore, they can be used to determine the total chemotherapy doses as basic parameters for evaluating relative benefits and hazard ratios. This evaluation of adjuvant therapies is of greatest importance, particularly because of the feasible practical application of this adjuvant therapy as a low-cost agent.

The limitations of study include short-term follow-up, and larger multicenter studies are warranted.

Low-dose oral MTX can be safely used in the treatment of patients with leukemia without the risk of significant fibrosis in the majority of patients. While we could not show the beneficial role of antioxidant agents, such as vitamin E or UDCA, in the primary or secondary prevention of MTX-induced liver damage.

Ethics Committee Approval: Ethics committee approval was received for this study from Ethics Committee of Shiraz University (Decision No: CT-P-9364-6404).

Informed Consent: Informed consent was obtained from patient's parent who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - M.R.B., N.H., N.S.H.; Design - M.R.B., N.H., N.S.H.; Supervision - M.R.B., N.H., M.R.F.; Resource - M.R.B., N.H., M.R.F.; Materials - M.R.B., N.H., S.H.; Data Collection and/or Processing - M.R.B., N.H., S.H.; Analysis and/or Interpretation - M.R.B., N.H., S.H.; Literature Search - M.R.B., N.H., S.H.; Writing - M.R.B., N.H., M.R.F.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Hamilton LA, Collins-Yoder A, Collins RE. Drug-Induced Liver Injury. AACN Adv Crit Care 2016; 27: 430-40. [CrossRef]

2. Teschke R, Danan G. Drug-induced liver injury: Is chronic liver disease a risk factor and a clinical issue? Expert Opin Drug Metab Toxicol 2017; 13: 425-38. [CrossRef]

3. Sharma A, Houshyar R, Bhosale P, Choi JI, Gulati R, Lall C. Chemotherapy induced liver abnormalities: an imaging perspective. Clin Mol Hepatol 2014; 20: 317-26. [CrossRef]

4. Conway R, Low C, Coughlan RJ, O'Donnell MJ, Carey JJ. Risk of liver injury among methotrexate users: A meta-analysis of randomised controlled trials. Semin Arthritis Rheum 2015; 45: 156-62. [CrossRef]

5. Elbarbary NS, Ismail EA, Farahat RK, El-Hamamsy M. ω-3 fatty acids as an adjuvant therapy ameliorates methotrexate-induced hepatotoxicity in children and adolescents with acute lymphoblastic leukemia: A randomized placebo-controlled study. Nutrition 2016; 32: 41-7. [CrossRef]

6. Famurewa AC, Ufebe OG, Egedigwe CA, Nwankwo OE, Obaje GS. Virgin coconut oil supplementation attenuates acute chemotherapy hepatotoxicity induced by anticancer drug methotrexate via inhibition of oxidative stress in rats. Biomed Pharmacother 2017; 87: 437-42. [CrossRef]

7. Al-Tonbary Y, Al-Haggar M, El-Ashry R, El-Dakroory S, Azzam H, Fouda A. Vitamin E and N-acetylcysteine as antioxidant adjuvant therapy in children with acute lymphoblastic leukemia. Adv Hematol 2009; 2009: 689639. [CrossRef]

8. Mohammed Saif M, Farid SF, Khaleel SA, Sabry NA, El-Sayed MH. Hepatoprotective efficacy of ursodeoxycholic acid in pediatrics

acute lymphoblastic leukemia. Pediatr Hematol Oncol 2012; 29: 627-32. **[CrossRef]**
9. Uraz S, Tahan V, Aygun C, et al. Role of ursodeoxycholic acid in prevention of methotrexate-induced liver toxicity. Dig Dis Sci 2008; 53: 1071-7. **[CrossRef]**
10. Baranova A, Lal P, Birerdinc A, Younossi ZM. Non-invasive markers for hepatic fibrosis. BMC Gastroenterol 2011; 11: 91. **[CrossRef]**
11. Webb M, Shibolet O, Halpern Z, et al. Assessment of liver and spleen stiffness in patients with myelofibrosis using fibroscan and shear wave elastography. Ultrasound Q 2015; 31: 166-9. **[CrossRef]**
12. Solá S, Aranha MM, Steer CJ, Rodrigues CM. Game and players: mitochondrial apoptosis and the therapeutic potential of ursodeoxycholic acid. Curr Issues Mol Biol 2007; 9:123-38.
13. Lapenna D, Ciofani G, Festi D, et al. Antioxidant properties of ursodeoxycholic acid. Biochem Pharmacol 2002; 64: 1661-7. **[CrossRef]**
14. Honar N, Ghashghaei Saadi E, Saki F, Pishva N, Shakibazad N, Hosseini Teshnizi S. Effect of ursodeoxycholic acid on indirect hyperbilirubinemia in neonates treated with phototherapy. J Pediatr Gastroenterol Nutr 2016; 62: 97-100. **[CrossRef]**
15. Abbas G, Lindor KD. Pharmacological treatment of biliary cirrhosis with ursodeoxycholic acid. Expert Opin Pharmacother 2010; 11: 387-92. **[CrossRef]**
16. Lindor K. Ursodeoxycholic acid for the treatment of primary biliary cirrhosis. N Engl J Med 2007; 357: 1524-9. **[CrossRef]**
17. Ladas EJ, Jacobson JS, Kennedy DD, Teel K, Fleischauer A, Kelly KM. Antioxidants and cancer therapy: a systematic review. J Clin Oncol 2004; 22: 517-28. **[CrossRef]**
18. Moss RW. Should patients undergoing chemotherapy and radiotherapy be prescribed antioxidants? Integr Cancer Ther 2006; 5: 63-82. **[CrossRef]**
19. Lawenda BD, Kelly KM, Ladas EJ, Sagar SM, Vickers A, Blumberg JB. Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? J Natl Cancer Inst 2008; 100: 773-83. **[CrossRef]**