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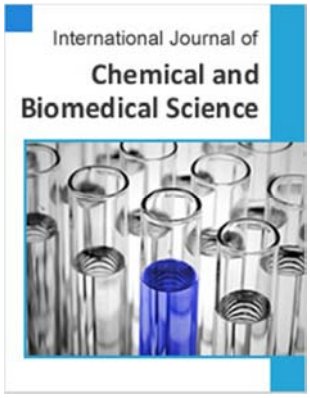

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Clinical Chemistry Studies in Egyptian Children with Acute Lymphoid and Myeloid Leukemia

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Abstract

Hepatotoxicity from chemotherapy occurs frequently. Methotrexate (MTX) was used as chemotherapy to treat children from leukemia (12 mg/dose intrathecally) (The dose may be administered every 2 to 5 days until CSF counts return to normal followed by one additional dose). Increases in aminotransferases (transaminitis) are potential major adverse reactions seen with long-term use of methotrexate (MTX). silymarin is liver support drug that has antioxidant activity, it promotes hepatocyte regeneration, reduces the inflammatory reaction, inhibits the fibrogenesis in the liver and protect from hepatotoxicity. Laboratory liver tests are broadly defined as tests useful in the evaluation and treatment of patients with hepatic dysfunction. in the present study. liver function tests were done to children with acute leukemia and healthy. A significant increased to total bilirubin, indirect bilirubin for children with leukemia during chemotherapy compared to their levels before chemotherapy and in healthy control. The results also revealed that, there were no significant differences on Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities between children with leukemia during and before chemotherapy compared with their activities in healthy control children. This study proved that there were liver abnormalities to children with leukemia that not significant in most tests due to using silymarin drug.

1. Introduction

Appelbaum et al., (2011); Kantarjian and O'Brien, (2011) reported that leukemia is a type of blood cancer that begins in the bone marrow. Leukemia leads to an uncontrolled increase in the number of white blood cells. The cancerous cells prevent healthy red cells, platelets, and mature white cells (leukocytes) from being made. The main leukemia types are: acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML).

Hepatotoxicity from chemotherapy occurs frequently from an unpredictable or idiosyncratic reaction. Common Toxicity Criteria for Adverse Events" has classified elevations of serum enzyme activities (alanine aminotransferase" (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyltransferase (GGT)) into mild (grade 1) if >ULN (upper limits of normal) to 2.5×ULN; moderate (grade 2)

if >2.5 to 5×ULN; severe (grade 3) if >5 to 20×ULN; and life-threatening (grade 4) if >20×ULN; and with no definition for fatal (grade 5). Similarly, they graded serum total bilirubin concentration as mild if >ULN to 1.5×ULN, moderate if >1.5 to 3×ULN, severe if >3 to 8×ULN, and life-threatening if >8×ULN (Alla and Christopher, 2014).

Methotrexate has been used in the treatment of certain chronic medical disorders e.g. rheumatoid arthritis and psoriasis as well as a number of malignant disorders e.g. acute lymphoblastic leukemia, certain types of lymphoma and breast carcinoma. Its use has been associated with various systemic toxicities and complications. (Khalid et al., 2009).

Methotrexate was used to treat cancer and can cause of the hepatotoxicity that lead to increasing transaminases, the administration of this drug was stopped and mitoxantrone was given instead. A recovery of clinical symptoms and normalisation of the liver function tests was observed afterwards.. An overview of the literature regarding methotrexate hepatotoxicity is presented (van Outryve et al., 2002).

The antileukemic mechanisms of 6-mercaptopurine (6MP) and methotrexate (MTX) maintenance therapy are poorly understood, but the benefits of several years of myelosuppressive maintenance therapy for acute lymphoblastic leukemia are well proven. The folate pathway gene expression profiles vary widely among subsets of ALL, which affects treatment efficacy of MTX. 6MP and MTX are hepatotoxic and 2-fold elevations or more of serum aminotransferases are frequent but usually normalize within a few weeks after discontinuation of maintenance therapy (Kjeld et al., 2014).

The liver is an important target of the toxicity of drugs, xenobiotics, and oxidative stress. In cholestatic disease, endogenously generated bile acids produce hepatocellular apoptosis by stimulating Fas translocation from the cytoplasm to the plasma membrane where self-aggregation occurs to trigger apoptosis. Kupffer cell activation and neutrophil infiltration extend toxic injury. Kupffer cells release reactive oxygen species (ROS), cytokines, and chemokines, which induce neutrophil extravasation and activation. The liver expresses many cytochrome P450 isoforms, including ethanol-induced CYP2E1. CYP2E1 generates ROS, activates many toxicologically important substrates, and may be the central pathway by which ethanol causes oxidative stress. In acetaminophen toxicity, nitric oxide (NO) scavenges superoxide to produce peroxynitrite, which then causes protein nitration and tissue injury. In inducible nitric oxide synthase (iNOS) knockout mice, nitration is prevented, but unscavenged superoxide production then causes toxic lipid peroxidation to occur instead. Microvesicular steatosis, nonalcoholic steatohepatitis (NASH), and cytolytic hepatitis involve mitochondrial dysfunction, including impairment of mitochondrial fatty acid beta-oxidation, inhibition of mitochondrial respiration, and damage to mitochondrial DNA. Induction of the mitochondrial permeability transition (MPT) is another mechanism causing mitochondrial failure, which can lead to necrosis from ATP depletion or caspase-dependent apoptosis if ATP depletion does not occur fully. Because of such diverse mechanisms, hepatotoxicity remains a major reason for drug withdrawal from pharmaceutical development and clinical use (Jaeschke et al., 2002).

The silymarin exerts membrane-stabilizing and antioxidant activity, it promotes hepatocyte regeneration; furthermore it reduces the inflammatory reaction, and inhibits the fibrogenesis in the liver. These results have been established by experimental and clinical trials. According to open studies the long-term administration of silymarin significantly increased survival time of patients with alcohol induced liver cirrhosis. Based on the results of studies using methods of molecular biology, silymarin can significantly reduce tumor cell proliferation, angiogenesis as well as insulin resistance. Furthermore, it exerts an anti-atherosclerotic effect, and suppresses tumor necrosis factor-alpha-induced protein production and mRNA expression due to adhesion molecules. The chemopreventive effect of silymarin on HCC has been established in several studies using in vitro and in vivo methods; it can exert a beneficial effect on the balance of cell survival and apoptosis by interfering cytokines. In addition to this, anti-inflammatory activity and inhibitory effect of silymarin on the development of metastases have also been detected. In some neoplastic diseases silymarin can be administered as adjuvant therapy as well (Féher and, Lengyel, 2012).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes found mainly in the liver, but also found in red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys. AST and ALT formerly are called serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), respectively. AST or ALT levels are a valuable aid primarily in the diagnosis of liver disease. Although not specific for liver disease, it can be used in combination with other enzymes to monitor the course of various liver disorders. When body tissue or an organ such as the liver or heart is diseased or damaged, additional AST and ALT are released into the bloodstream, causing levels of the enzyme to rise. Therefore, the amount of AST and ALT in the blood is directly related to the extent of the tissue damage. After severe damage, AST levels rise 10 to 20 times and greater than normal, whereas ALT can reach higher levels (up to 50 times greater than normal). On the other hand, the ratio of AST to ALT (AST/ALT) sometimes can help determine whether the liver or another organ has been damaged (Xing-Jiu et al., 2006).

Jaundice is a yellow color of the skin, mucus membranes, or eyes. The yellow coloring comes from bilirubin, a byproduct of old red blood cells. Jaundice can be a symptom of other health problems. Small number of red blood cells in your body die each day, and are replaced by new ones. The liver removes the old blood cells. This creates bilirubin. The

metabolism of the bilirubin illustrated in Fig. 1. The liver helps break down bilirubin so that it can be removed by the body in the stool. Jaundice can occur when too much bilirubin builds up in the body. Jaundice can occur if:, Too many red blood cells are dying or breaking down and going to the liver, The liver is overloaded or damaged or the bilirubin from the liver is unable to move into the digestive tract properly. Jaundice is often a sign of a problem with the liver, gallbladder, or pancreas. Things that can cause jaundice include: Infections, Use of certain drugs cancer, Blood disorders, gallstones, birth defects and a number of other medical conditions can lead to jaundice (Lidofsky, 2010).

Beckingham and Ryder, (2001) reported that types of jaundice are:

a. Pre-hepatic jaundice, in pre-hepatic jaundice, excess unconjugated bilirubin is produced faster than the liver is able to conjugate it for excretion. It is most commonly due to increased haemolysis for example, in spherocytosis, homozygous sickle cell disease, or thalassaemia major and patients are often anaemic with splenomegaly
b. Hepatic jaundice, that the most common intrahepatic causes are viral hepatitis, alcoholic cirrhosis, primary biliary cirrhosis, drug induced jaundice, and alcoholic hepatitis
c. Post-hepatic jaundice that is most often due to biliary obstruction by a stone in the common bile duct or by carcinoma of the pancreas. In obstructive jaundice, the serum bilirubin is principally conjugated. Diagnostic tests of jaundice illustrated in Table 1.

Liver involvement with AML is rarely reported. The majority of published cases suggest a cholestatic picture and obstructive jaundice at presentation. The author reported also elevated liver function tests persisted in his study despite cholecystectomy, however, they normalized with chemotherapy administration suggesting that AML was the causative effect of the hepatitis-like picture (Emily et al., 2008).

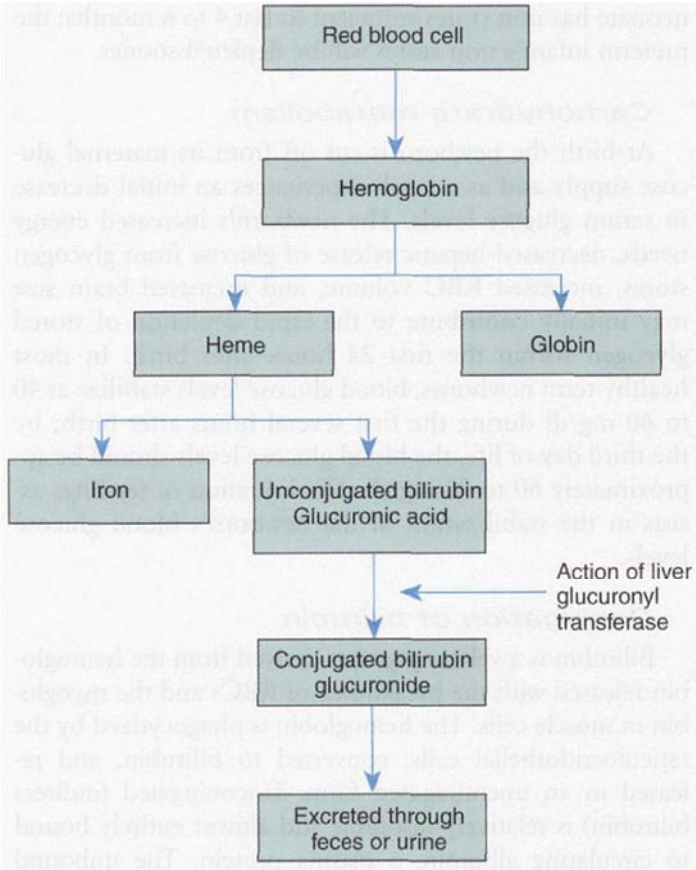

Fig. 1. *Bilirubin formation (Wong, 1999)*

Table 1. *Diagnostic tests of jaundice (Goljan, 2007).*

Table of diagnostic tests			
Function test	**Pre-hepatic jaundice**	**Hepatic jaundice**	**Post-hepatic jaundice**
Total bilirubin	Normal / increased	Increased	
Conjugated bilirubin	Normal	Increased	
Unconjugated bilirubin	Normal / increased	Increased	Normal
Alkaline phosphatase levels	Normal	Increased	
Alanine transferase and aspartate transferase levels		Increased	

2. Material and Methods

2.1. Sampling

Blood samples were taken from patients and healthy children at the Hematology & Oncology unit, pediatric hospital, Ain Shams University and Pediatric Department, Tanta Cancer Center.

2.2. Laboratory Equipments

1. Micopette pipettes, Dragon Lab Company, China.
2. Prietest Eco Automatic Biochemistry Analyzer, Robonik Company, India.
3. Centrifuge Model 800, Xiangshui Fada Medical Apparatus factory, China.
4. BT1020 – Medical Incubator, BioTech Company, Egypt.
5. Alanine Aminotransferase (ALT/GPT) reagent, Biosystems, Costa Brava, 30. 08030 Barcelona (Spain).
6. Aspartate Aminotansferase (AST/GOT) reagent, Biosystems, Costa Brava, 30. 08030 Barcelona (Spain).
7. Bilirubin reagents, Diamond Diagnostics, 23 El-Montazh st, Heliopolis, Cairo, Egypt

2.3. Patients and Controls

a. This study was done according to guidelines of Egyptian minister of health and population decree 95/year 2005 for medical research, good clinical practice, Declaration of Helsinki and World Health Organization Guidelines
b. This study included
   1. Group I: included 30 patients with acute leukemia (5-15 years old) (27 acute lymphoid leukemia + 3 acute myeloid leukemia) and classified into:
c. Group Ia: included 9 children with leukemia newly diagnosed before chemotherapy.
d. Group Ib: included 21 children with leukemia during chemotherapy.
   2. Group II: included 20 healthy control children with the same age range of children with leukemia.

2.4. Measuring of Liver Function Tests

2.4.1. Determination of Alanine Aminotransferase (ALT/GPT) (Gella et al., 1985)

Reagents composition:

A. Reagent: Tris 150 mmol/L, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L, pH 7.3.

B. Reagent: NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.

Principle of the method:

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm; by means of the lactate dehydrogenase (LDH) coupled reaction

$$\text{Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALE}} \text{pyruvate} + \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

Clinical significance:

The aminoatransferases catalyze the formation of glutamic acid from 2-oxoglutarate by transfer of amino groups. ALT is normally present in various tissues but its higher concentrations are found in liver and kidney. The serum concentration of ALT is elevated in hepatitis and other forms of hepatic disease associated with necrosis: infectious mononucleosis, cholestasis, cirrhosis, metastasic carcinoma of the liver, delirium tremens, and after administration of various drugs, such as opiates, salycilates or ampicillin. Serum ALT concentration can also be elevated in skeletal or cardiac muscle disease. Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

Procedure:

1. Bring the working reagent and the instrument to reaction temperature.
2. Pipette into a cuvette:

Reaction temperature	37°C	30°C
Working Reagent	1 ml	1 ml
Sample	50 µL	100 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 1 minute, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (DA/min).

2.4.2. Determination of Aspartate Aminotransferase (AST/GOT) (Gella et al., 1985)

Principle of the method:

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+$$

Clinical significance:

The aminotransferases catalyze the formation of glutamic acid from 2-oxoglutarate by transfer of amino groups. AST is found in highest concentration in the liver and heart muscle but it is also abundant in skeletal muscle, kidney

and pancreas. The serum concentration of AST is elevated in hepatitis and other forms of hepatic disease associated with necrosis: infectious mononucleosis, cholestasis, cirrhosis, metastasic carcinoma of the liver, delirium tremens, and after administration of various drugs. Serum AST concentration is also elevated after myocardial infarction, in skeletal muscle disease (as progressive muscular distrophy), in acute pancreatitis or hemolytic disease and other. Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

Reagents composition:

A. Reagent: Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase > 460 U/L, lactate dehydrogenase > 660 U/L, Sodium hydroxide 255 mmol/L, pH 7.8.

B. Reagent: NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L

Procedure:

1. Bring the Working Reagent and the instrument to reaction temperature.

2. Pipette into a cuvette:

Reaction temperature	37°C	30°C
Working Reagent	1 ml	1 ml
Sample	50 µL	100 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.

4. After 1 minute, record initial absorbance and at 1 minute intervals thereafter for 3minutes.

5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (DA/min).

2.4.3. Determination of Bilirubin (Tietz et al., 1995)

Principle of the method:

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. of the two presents in serum, bilirubin –glucuromide and free bilirubin loosely bound to albumin, only the former reacts directly in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with caffeine to react (bilirubin indirect). In the determination, the results correspond to total bilirubin. The intensity of the color formed is proportional to the bilirubin concentration in the sample.

Clinical significance:

Bilirubin is a breakdown product of hemoglobin. it is transported from the spleen to the liver and excreted into bile. Hyperbilirubinemia results from the increase of bilirubin concentrations in plasma.

Causes of hyperbilirubinemia:

a. Total bilirubin: increased hemoloysis, genetic errors, neonatal jaundice, ineffective erythropoiesis, drugs and reticulocytosis.

b. Direct bilirubin: Hepatic cholestasis, genetic errors, hepatocellular damage Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Reagents composition:

Reagent1	Hydrochloric Acid 23%
Reagent 2	Sodium Nitrate
Reagent 3	Caffeine, sodium benzoate
Reagent 4	Tartate, NaOH

Procedure:

Wavelength: Total bilirubin 578 nm & Direct bilirubin 546 nm

Optical path 1 cm & Incubation temperature 20 – 25°C

Zero adjustment specimens blank

Total bilirubin:

	Specimen blank	Specimen
R1	0.2 ml	0.2 ml
R2	----	1 drop
R3	1 ml	0.2 ml
Specimen	0.2 ml	0.2 ml
Mix and incubate 10 min at 20-25 C then add		
R4	1 ml	1 ml
Mix and incubate for 5 minutes at 20 -25 c. Read absorbance of specimen against specimen blank		

Direct Bilirubin:

	Specimen blank	Specimen
R1	2 ml	0.2 ml
R2	----	1 drop
NaCl (0.9%)	2 ml	2 ml
Specimen	0.2 ml	0.2

Mix and incubate for exactly 5 minutes at 20 -25 c. Read absorbance of specimen against specimen blank.

3. Result

3.1. Determination of Serum SGPT/ALT Activity

The results in Table 2 showed that the activity of ALT (SGPT) before chemotherapy were ranging from 21 U/L to 39 U/L but during chemotherapy were ranging from 25 U/L to 61 U/L and in healthy control were ranging from 18 U/L to 37 U/L. The present study revealed that; SGPT elevated in 33.3% patients with leukemia (10% before chemotherapy and 23.3% during chemotherapy). The results also revealed that, there were no significant differences on SGPT activities between children with leukemia during and before chemotherapy compared with their activities in healthy control children (P value =0.065).

Table 2. *Comparison between children with leukemia before and during chemotherapy referred to healthy control children as regards serum SGPT/ALT activity.*

Groups	ALT (SGPT) by (U/L)						ANOVA	
	Range by U/L			Mean	±	SD	F	P-value
Before chemotherapy	21	-	39	32.777	±	5.517		
During chemotherapy	25	-	61	35.265	±	9.594	2.888	0.065
Healthy control	18	-	37	29.77	±	4.783		

Normal values of serum SGPT are up to 40 U/L

3.2. Determination of Serum SGOT/AST Activity

The results in Table 3 showed that the activity of AST (SGOT) before chemotherapy were ranging from 19 U/L to 35 U/L but during chemotherapy were ranging from 24 U/L to 49 U/L and in healthy control were ranging from 17 U/L to 34 U/L. The present study revealed that; SGOT elevated in 20% patients with leukemia (3.3% chemotherapy and 16.7% during chemotherapy). The results also revealed that, there were no significant differences on SGOT activities between children with leukemia during and before chemotherapy compared with their activities in healthy control children (P value =0.072).

Table 3. *Comparison between children with leukemia before and during chemotherapy referred to healthy control children as regards serum SGOT / AST activity.*

Groups	AST (SGOT) by (U/L)						ANOVA	
	Range by U/L			Mean	±	SD	F	P-value
Before chemotherapy	19	-	35	29.111	±	4.935		
During Chemotherapy	24	-	49	32.341	±	6.707	2.771	0.072
Healthy Control	17	-	34	28.480	±	4.065		

Normal values of serum SGOT are up to 40 U/L

3.3. Determination of Serum Total Bilirubin

The results in Table 4 showed that the levels of total bilirubin before chemotherapy were ranging from 0.55 mg/dl to 1.07 mg/dl but during chemotherapy were ranging from 0.52 mg/dl to 1.7 mg/dl and in healthy control were ranging from 0.5 mg/dl to 1 mg/dl. The results of total bilirubin were significant increased during chemotherapy compared to their levels before chemotherapy and in healthy control (P value =0.023) (P value =0.019) respectively.

Table 4. *Comparison between children with leukemia before and during chemotherapy referred to healthy control children as regards serum total bilirubin.*

Groups	Total bilirubin by (mg/dl)						ANOVA	
	Range by mg/dl			Mean	±	SD	F	P-value
Before chemotherapy	0.55	-	1.07	0.722	±	0.165		
During chemotherapy	0.52	-	1.7	0.987	±	0.332	5.544	0.006*
Healthy Control	0.50	-	1.00	0.774	±	0.133		
TUKEY'S Test								
Before chemotherapy & During chemotherapy				Before chemotherapy & Healthy control			During chemotherapy & Healthy control	
0.023*				0.854			0.019*	

Normal values of serum total bilirubin are up to 1.2 mg/dl

3.4. Determination of Serum Direct Bilirubin

The results in Table 5 showed that the levels of direct bilirubin before chemotherapy were ranging from 0.11 mg/dl to 0.21 mg/dl, during chemotherapy were ranging from 0.11 mg/dl to 0.40 mg/dl but in healthy control were ranging from 0.1 mg/dl to 0.25 mg/dl. The results also revealed that, there were no significant differences in levels of serum direct bilirubin between children with leukemia before and during chemotherapy compared to their levels in healthy control children (P value = 0.109).

Table 5. *Comparison between children with leukemia before and during chemotherapy referred to healthy control children as regards serum direct bilirubin.*

Groups	Direct bilirubin by (mg/dl)						ANOVA	
	Range by mg/dl			Mean	±	SD	F	P-value
Before chemotherapy	0.11	-	0.21	0.151	±	0.036		
During chemotherapy	0.11	-	0.40	0.202	±	0.068	2.320	0.109
Healthy control	0.1	-	0.25	0.179	±	0.051		

Normal values of serum direct bilirubin are up to 0.25 mg/dl

3.5. Determination of Serum Indirect Bilirubin

The results in Table 6 showed that the levels of indirect bilirubin before chemotherapy were ranging from 0.43 mg/dl to 0.86 mg/dl but during chemotherapy were ranging from 0.41 mg/dl to 1.4 mg/dl and in healthy control were ranging from 0.25 mg/dl to 81 mg/dl. The results of indirect bilirubin were significant increased during chemotherapy compared to their levels before chemotherapy and in healthy control (P value =0.036), (P value =0.023) respectively.

Table 6. *Comparison between children with leukemia before and during chemotherapy referred to healthy control children as regards serum indirect bilirubin.*

Groups	Indirect bilirubin by (mg/dl)						ANOVA	
	Range by mg/dl			Mean	±	SD	F	P-value
Before chemotherapy	0.43	-	0.86	0.571	±	0.141		
During chemotherapy	0.41	-	1.4	0.785	±	0.276	5.065	0.010*
Healthycontrol	0.25	-	0.81	0.605	±	0.144		
TUKEY'S Test								
Before chemotherapy & During chemotherapy			Before chemotherapy & Healthy control			During chemotherapy & Healthy control		
0.036*			0.912			0.023*		

Normal values of serum indirect bilirubin are up to 0.95 mg/dl

4. Discussion

In the present work, the obtained results showed that there were no significance differences in the activity of liver enzymes (SGPT, SGOT) between children with leukemia before and during chemotherapy compared to healthy control children. This was because of those children had silymarin drug as liver support drug to help in filtering the toxicity action of chemotherapy. Also results showed significance increase in levels of serum total bilirubin and indirect bilirubin for children with leukemia during chemotherapy compared with their levels before chemotherapy and in healthy control however no significance difference was showed in levels of serum direct bilirubin between children with leukemia before and during chemotherapy compared with healthy control children. This may due to pre-hepatic jaundice because of hemolytic anemia as result of action chemotherapy (Woolley et al., 1983)

The present study revealed that; SGPT elevated in 16.7% patients with leukemia during chemotherapy, SGOT elevated in 10% patients with leukemia during chemotherapy, total bilirubin elevated in 13.3% patients with leukemia during chemotherapy, indirect bilirubin elevated in 13.3% patients with leukemia during chemotherapy and direct bilirubin elevated in 10% patients with leukemia during chemotherapy. The study revealed that there were two cases of sepsis, the two cases were during chemotherapy and showed elevations in levels of SGOT, SGPT, total bilirubin, direct bilirubin and indirect bilirubin. All previous elevations in liver function tests due to leukemia itself or chemotherapy or infections

In the same way of Sharma and Karki, (2007) studied abnormal hepatic functions on the newly diagnosed acute leukemia patients. They reported that twelve (7.59%) patients presented with hepatomegaly. Serum ALT was elevated in 54 (34.17%) patients. Similarly, serum AST, GGT, ALP, and Direct bilirubin were elevated in 26 (16.45%), 32 (20.25%), 20 (12.65%), and 22 (13.92%) patients, respectively. Also the results similar with Yukihiro, (2008) reported that hepatic involvement in acute leukemia is usually mild and silent at the time of diagnosis, a post mortem study showed liver infiltration in > 95% of ALL and up to 75% of AML patients. In patients with acute leukemia, drug-induced liver injury and bacterial or fungal infections may also affect the liver and Locasciulli et al. (1992) studied high-dose methotrexate administration and acute liver damage in children treated for acute lymphoblastic leukemia. They reported that prevalence of high-dose methotrexate (HDMTX)-induced acute hepatotoxicity was 1.47% (1/68 patients).

On the same line, Segal et al. (2010) studied abnormal liver transaminases and conjugated hyperbilirubinemia at presentation of acute lymphoblastic leukemia. They reported that one hundred forty-seven ALL patients were identified. Over one third of patients had abnormal liver transaminase values (AST and/or ALT). Of the patients with abnormal transaminases, (52%) had ALT elevations twice the upper limit of normal. Risk factors for elevated transaminases included a high WBC count at diagnosis, older age, bulky disease, and T-cell leukemia. Conjugated hyperbilirubinemia was observed in 3.4% of subjects. Of these cases, 60% received steroids prior to induction chemotherapy and all had rapid resolution of their hyperbilirubinemia to normal levels.

Shah et al. (2010) reported that the common conditions that resulted in elevated liver enzymes were sepsis. All patients with elevated parameters of liver function tests (LFTs) were fully investigated, managed and followed up in accordance with american gastroenterology association (AGA) guidelines. In addition, in patients with bacterial sepsis, old age was associated with increased mortality, while development of jaundice in elderly patients with bacterial sepsis was associated with increased survival

5. Conclusion

The thrust of this study to measure serum bilirubin, ALT activity and AST activity for evaluation the effect of methotrexate on liver. This study proved that there were liver abnormalities to children with leukemia that not significant in most tests due to using silymarin drug. Silymarin is good liver support that can detoxify the liver from side effects of methotrexate

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