

Study of Some Biochemical Parameters in Patients of Chronic Lymphocytic Leukaemia (CLL)

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الخلاصة:

تم فحص (25) عينة دم من مرضى مصابين بسرطان الخلايا اللمفاوية المزمن قبل وبعد علاجهم بالكيميائي في المركز الوطنى لبحوث وعلاج امراض الدم للفترة من (شباط الى تشرين الثاني) عام 2004 وقد تم تشخيص المرضى طبقاً للعلامات السريرية والفحوصات الدموية وكذلك تشخيص معلمات الخلايا السرطانية بطريقة الفلورسانت مايكرو سكوب حيث كان معدل اعمار المصابين (50,46) ولم تكن هناك حالة اقل من (40) عام تم تقييم وظائف الكبد ووجد ارتفاع معدلات الانزيمات المفترزة من قبل الكبد عن المرضى.

Summary:

Twenty five samples were collected from patients with chronic lymphoid leukaemia (CLL) before chemotherapy and after chemotherapy in national center of haematology from the period began at February till the end November 2004.

The diagnosis was established depending on clinical and hematological findings and immunopheno typing performed by immunofluorescence technique (fluorescent microscopy) the 25 cases of CLL patients included in this study had mean age of 50.46± 9.34 years and no case below the age of 40 years.

The study evaluated the liver function, the abnormalities in liver function and hepatotoxicity in the patients as result of chemotherapy, deviation in glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT) in 100% of patients also elevated of total serum bilirubin (TSB) in patients but mild elevation in alkaline phosphates (ALT).

Introduction:

Chronic lymphoproliferative disorders (CLPDs) are a heterogeneous complex group of disease derived from neoplastic clonal proliferation of cytological LLy immunophenotypically mature B or T cells^[1,2], the disorders that predominantly affect lymph nodes other extramedullary sites are generally regarded as lymphoma whereas disease with predominant bone marrow blood manifestation are generally labeled as leukaemia.

CLL: Are clonal malignancies resulting from expansion of mature looking lymphocytes as consequence of prolonged cell survival, despite low proliferative index^[3,4].

Materials and Methods:

Instrument and equipments:

Instruments	Company	Source
Spectrophotometer	Ceilceololl	France
Centrifuge	Griffin	U.K
Incubator	Fisher	Scientific Turkish
Micropipette	Oxford pipette	France
Magnetic stirrer	Baind tat lock	
Water bath	Memert	

Biochemical kits:

Kit	company	source
Alkaline phosphatase	Biomerieux	France
Enzyme	Biomerieux	France
GOT	Biomerieux	France
GPT	Biomerieux	France
TSB	Biomerieux	France

Samples:

Blood samples were collected from (25) patients and 20 healthy person from the national center of hematology these (25) patients with CLL.

Procedure:

Determination of total bilirubin (T.S.B):

- 1- Reagent composition RT = sulfanilic acid 29 mol /l+hydrochloric acid 0.20mol/L Sodium nitrate 11.6 m mol/l Bilirubin + Diazotized sulfanilic acid. Azobilirubin measured photo metrically.
- 2 - Working reagent (WR) is prepared 1 volume of RN+4 volume of RT.

Procedure:

- 1)
 - A - Reagent blank 100 ml DW+1.0 ml working reagent.
 - B - Sample blank 100ml serum+1.0ml working reagent.
 - C - Sample 100ml serum+1.0 ml WR.
 - D - Standard 100 ml of standard solution +10ml of RT.
- 2) Mix thoroughly and let the tube stand for 2 minute at room temperature.
- 3) Read the absorbance (A) of the sample blank at 540 nm against (DW).
- 4) Read the absorbance (A) of the sample at 540 nm against the reagent blank.

Calculation:

Sample A – Sample A blank

$$\frac{\text{Sample A} - \text{Sample A blank}}{\text{Standard A}} \times C \text{ standard} = \text{mg/dl}$$

Serum glutamate oxaloacetate transaminase (SGOT) enzyme activity determination:

(Rietman and Frankel method)

The substrate of enzyme Aspartate+ a ketoglutarate after incubation at 37°C with enzyme for 1 hour exactly the produced reaction oxalo acetate+glutamate

GOT:

Aspartate + a ketoglutarate ----- Oxalo acetate+glutamate

The PH of reaction is 7.5 and optimum temperature 37°C

Oxalo acetate formed is measured in its derivatives from 2.4 Dinitrophenel hydrozone

Methods

Procedure:

- 1 - Included the substrate for 5 minutes at 37°C with buffer solution
- 2 - Add the enzyme to the substrate and incubate for 1 hour at 37°C
- 3 - Add the color reagent and wait for 20 minutes
- 4 - Add NAOH 0.4 N
- 5 - Absorbance was measured and determined the activity of the enzyme determined u/ml from stranded curve at wave 505 nm

Serum glutamate pyruvate transaminase serum (GPT) activities determination Rietman-Franke method:

The substrate of the enzyme Alanine + a ketoglutarate after incubation at 37 C° for 1 hour exactly the product of reaction is private + glutamate.

Alanine + a Ketoglutarate - pyrovate+glutamate the pyruvate will react with color reagent 2.4- Dinitro phenel hydrazine to produce a color solution then the absorbance is measured at 505nm

Procedure:

- 1- Incubate the substrate with buffer solution at 37°C 5 min to get the reaction temperature
- 2 - The enzyme was added and incubated for 1 hour exactly
- 3 - The color reagent was added 2-4 dinitrophenyle hydrazine
- 4 - To complete the reaction wait for 20 minutes
- 5 - Stop the reaction by adding 0.4 NAOH

6 - Wait 5 minutes and read the absorbance were measured and then the activity calculated

Alkaline phosphatase enzyme (ALP) determination:

The substrate of the enzyme is the phenol phosphate the enzyme catalyzes the reaction to convert the substrate to phenol+phosphate pH 10 and temperature at 37°C

Liberated from the reaction and the amount indicates the activity of enzyme at 15 minutes. Liberated phenol reacts with 4-amino antipyrine and potassium ferric cyanide colored the solution. Absorbance measured at 510nm, and then sodium arsenate stops the enzymatic reaction.

Procedure:

- 1- Serum sample and buffer substrate solution were incubated for 5 minutes at 37°C to get the temperature then serum was added
- 2- Incubated for 15 minutes at 37°C
- 3- 4-amino antipyrine+sodium arsenate+potassium ferric cyanide were added (color-solution) let stand for 5 minutes at dark measure the absorbance at 510 nm

O D serum- O D serum blank
Activity of enzyme = $\frac{X - n}{I} = U/I$
O D standard

Result:

This study included 25 patients with chronic lymphoid leukaemia (CLL) before and after treatment. Result in table (1) figure (1) show the mean value of total serum bilirubin for (CLL) patients, before chem. Therapy (1.48 mg/dl) while after treatment the value increase to (1.525 mg/dl) but the normal value (0.697 mg/dl)

Table (2) figure (2) shows the mean activity value of ALP enzyme for (CLL) patients before treatment (9.9 u/dl) while the normal value (7.0u/dl) and after treatment the value become (10.7u/dl)

Table (3) figure (3) shows the mean values of GPT enzyme activity for (CLL) patients was significantly elevated before treatment (48.75 u/ml) compared with normal values (31.005 u/ml) after treatment (56.57 u/ml)

Table (4) figure (4) show the mean value of GOT enzyme for groups of (CLL) patients before treatment (154.102 u/ml) and after treatment (144.35 u/ml) compared with the normal value of (31.62 u/ml)

Discussion:

Evaluation of some biochemical parameters for patients of (CLL) were carried out monitoring the elevation of these values .Data in table (1) figure (1)

shows the values of total serum bilirubin before and after treatment when compared with values of control. It was found that hyperbilirubinemia may be red cells after hemolysis or break down of the red cells after hemorrhage ^[5].

TSB also elevated due chemotherapy and causes increase in the toxicity of the liver cells which causes hepatomegaly as total serum bilirubin deviated more than the case before treatment (AMA) council report 1985 ^[6], also development of secondary sclerosing cholangitis ^[7,8].

Liver serves many vital functions in the body: the most important function is to filter toxic substance from blood if there are more toxins coming the liver can deal with.

The liver is most important task is to filter toxic substances from body, including drugs in chemotherapy if toxins accumulate in the body faster than the liver can process them, the liver damage will result ^[9].

Table (3) figure (3) table (4) figure (4) show elevation in transaminase enzyme activity in all patients this elevation related to many reasons, as result of toxicity of liver cells which lead to the damage of liver cells, detoxification of toxin depends on the Kupffer cells which are mostly damaged ^[10] liver dysfunction is common in patients with cancer, serum transaminase ace GOT, GPT increase more than normal ^[11].

Table (2) figure (2) shows elevation before treatment liver dysfunction is common in patients of cancer either due to hepatic metastasis as is order to liver impairment or toxicity of liver cells hypoalbuminemia which may be a consequence of liver impairment, can affect protein binding ^[12] drug of cytotoxic in patients with impaired liver the value of ALP increase is still less than expected and this may be related to the environmental conditions of nutrition immunity and genetic factor of Iraqi patients.

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Table(1) Descriptive statistic with their comparison of TSB mg/dl among different groups of CLL

Parameter	Statistics	Before treatment	Normal	After treatment	
TSB	Mean	1.4882	0.6975	1.5255	
	S.D	1.05122	0.00979	1.07204	
	95% C.I	U.B	1.98019	0.743	2.02723
		L.B	0.996	0.6517	1.02377

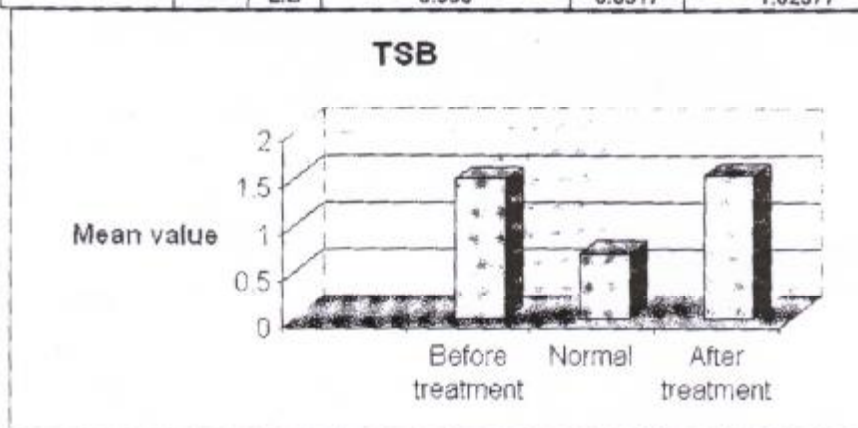


Figure (1) TSB parameter distribution CLL

Table(2) Descriptive statistic with their comparison of ALP u/dl among different groups of CLL

Parameter	Statistics	Before treatment	Normal	After treatment	
ALPM3	Mean	9.911	7.94	10.7665	
	S.D	3.5473	1.871	7.71	
	95%c.i	U.B	11.5707	8.816	14.376
		L.B	8.25	7.0644	7.158

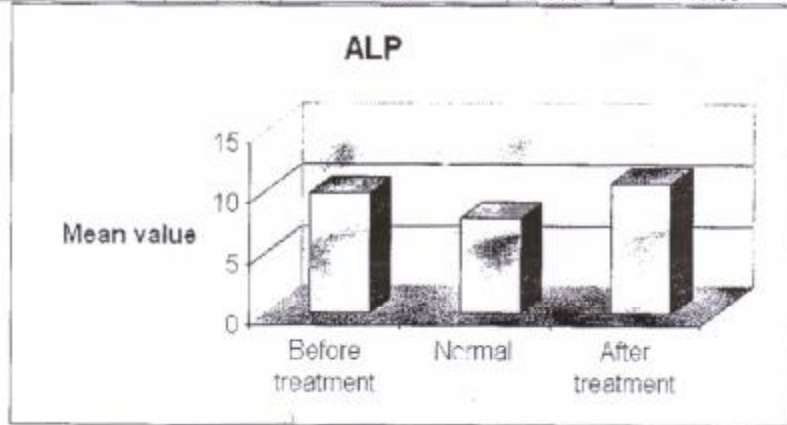


Figure (2) ALP parameter distribution

Table/3 Descriptive statistic with their comparison of GPT u/ml among different groups of CLL

Parameter	Statistics	Before treatment	Normal	After treatment	
GPT	Mean	48.75	31.005	56.575	
	S.D	12.196	4.384	24.07	
	95%c.i	U.B	54.458	33.057	67.84
		L.B	43.042	28.953	45.31

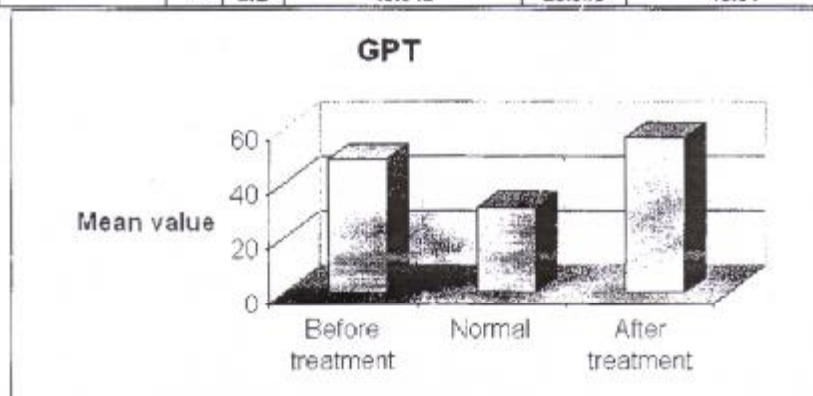


Figure (3) GPT parameter distribution.

Table 4 | Descriptive statistic with their comparison of GOT u/ml among different groups of CLL

Parameter	Statistics	Before treatment	Normal	After treatment	
GOT	Mean	154.102	31.625	144.35	
	S.D	25.768	3.83	25.274	
	95% c.I	U.B	166.162	33.417	156.179
		L.B	142.042	29.833	132.521

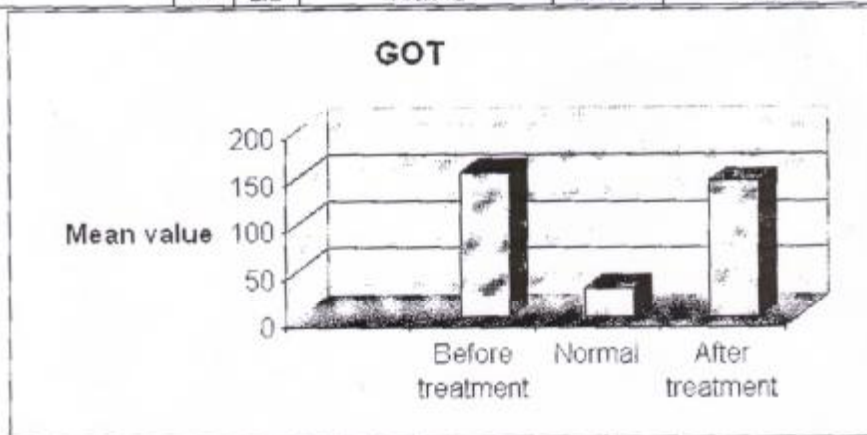


Figure 4 | GOT parameter distribution