ORIGINAL ARTICLE

Biochemistry of Various Ingredients in sera leukemic and Hodgkin's Patients

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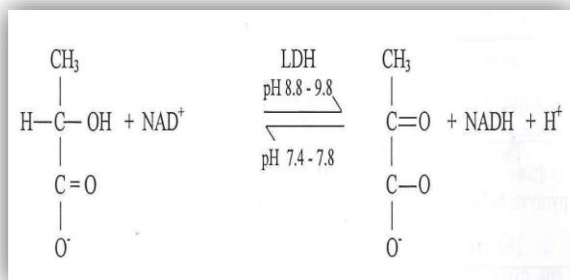
ABSTRACT

The present study was aimed to characterize the activity of lactate dehydrogenase and superoxide dismutase in sera patients with leukemia, Hodgkin's and non-malignant disease. The results shows an elevation in LDH activity in leukemia Hodgkin's and non-malignant, whereas the SOD activity in sera leukemia. Hodgkin's were decreased. The LASA/SOD ratio was also studied and found to be higher in leukemia, Hodgkin's patients. The results indicate that the SOD and LASA appear to be a suggested marker of leukemia and Hodgkin's disease. The trace elements level in sera leukemia and Hodgkin's were also carried out using atomic absorption and found to have an elevation in Cu and Cu/Zn ratio and Fe levels were decreased while Mg and Ca remain in normal range.
Keywords: Hodgkin's, Leukemia, biochemistry, ingredients

INTRODUCTION

Lactate dehydrogenase (LDH) is a hydrogen transfer enzyme that catalyzes the oxidation of lactate to pyruvate with the meditation of NAD^+ as hydrogen acceptor. The reaction is freely reversible[1].



The LDH is one of the non-plasma specific enzymes. The concentration of this enzyme in tissues is very high compared with those in sera, and its tissue level is about 500 times greater than those normally found in serum, and the leakage of the enzyme from even small mass damage tissue can increase the observed serum level of LDH to a significant extent. The elevation of lactate dehydrogenase activity in malignancy is non-specific; it has been demonstrated in a variety of cancer, including liver, non-Hodgkin's lymphoma acute leukemia and other cancers, such as breast, colon, stomach and lung cancer. An increase in serum LDH activity occurs in about 2/3 patients with leukemia and those with solid tumors, particularly wide spread or rapidly growing. In leukemia, the serum LDH levels are correlated with higher initial leukocyte counts but not with treatment outcome. Recently LDH level is associated with other parameter in monitoring the response to chemotherapy, irradiation therapy and bone marrow transplantation[2].

Superoxide dismutase SOD is an enzyme that catalyzes the breakdown of the oxygen and plays an important role in protecting the cells from damage through highly superoxide free radical. SOD can be found in all oxygen metabolizing systems and also in most oxygen tolerant organisms. It is particularly formed in the red cells and has been showed to be produced when oxyhemoglobin is autoxidezed to methemoglobin[3].

Superoxide is formed by the one electron reduction of oxygen, and has been identified as a product in a number of biological reactions, particularly in forming the red cell to produce the oxy-hemoglobin which autoxidezed to methemoglobin[4].

Other likely sources include reactions initiated by ionizing radiation. Superoxide dismutase was used to show that the oxidation of epinephrine to adenochrome by xanthine oxidase is mediated by the superoxide radical. Decreased activity of the enzyme superoxide dismutase (SOD) has also been found in all malignant tumors investigated so far. Such changes in the contact of glycolipids (Lipid-Associated Sialic Acid LASA) and the activity of SOD are not found in patient with benign tumors[5].

Although many details about the function of trace elements are not understood, some general characteristics are well known. These are amplification of trace element action, specificity, homeostasis, and interactions. The trace elements occur primarily in combination with proteins and are also frequently considered separately. The action of trace element is necessary for optimal performance of the whole organism. Lack of a small amount of trace (e.g., iron) can result in clinical abnormalities (anemia), seemingly disproportionate to the amount of element missing. The basis for this amplification of trace element action is constituents, with enzymes and hormones that regulate the metabolism of much larger amount of biochemical substance[6].

The present study was carried out to compare the content of LASA and activity of SOD in sera of patients with leukemia, Hodgkin's and the normal healthy individual.

MATERIALS AND METHODS

Chemicals: All common laboratory chemicals or reagents were of analar grade or equivalent, and were used without any further purification.

Instruments: LKB Spectrophotometer ultra spec type 4050, IR-Spectrometer type SP-3-300S.

Preparation of solutions

Buffers and substrate

1- Working tris buffer (0.056 M): 6.8 gm of tris (hydroxyl methyl) amino methane $[NH_2\text{-}C(CH_2\text{-}OH)_3]$ is dissolved in approximately 800 ml of distilled water, the pH is adjusted to 7.4 with HCl and the volume of the solution is brought to 1 Liter by distilled water. The solution is stable for 6 weeks at 4 °C.

2- Tris-NADH Reagent: 13 mg of NADH is dissolved in 90 ml of working tris-buffer. The absorbance is measured and brought to an absorbance of 1.0 (161 mM) by dilution with the same buffer, this solution is stable for 72 hours at 4 °C.

3- Pyruvate working solution (13.5 mM): 149 mg of sodium pyruvate is dissolved in 100 ml of distilled water. This solution is stable for 20 days at 4 °C.

4- Phosphate buffer (0.067 M), pH 7.8.

5- EDTA solution (0.1 M), containing 1.5 mg KCN per 100 ml.

6- Riboflavin solution (0.12 M) 4.5 mg of riboflavin dissolved in 100 ml of distilled water and stored in a dark bottle at 4 °C.

7- **NBT solution (1.5 mM):** 12.3 mg NBT dissolved in 100 ml of distilled water and stored at 4 °C.

Patients and blood samples

Sixty samples of blood from leukemia and Hodgkin's disease (16 ALL, 15 CML, 10 CLL, 5 AML, and 13 Hodgkin's), 7 with non malignant disease (as a control) and 6 normal individuals were collected from these patients, left for 30 minutes at room temperature, blood clots were separated using centrifuge at 3000 rpm for 10 minutes.

Determination of LDH activity in sera of Leukemia and Hodgkin's disease

1- 2 ml of tris-NADH reagent is placed in 3 ml cuvette.

2- 50 µl of sera was added, the solution was mixed thoroughly, and incubated for 10 minutes at 37 °C.

3- The reaction was initiated by adding of 0.1 ml working pyruvate solution. After mixing it, the cuvette is rapidly inserted into the spectrophotometer and the change in the absorbance at 340 nm was immediately measured.

The international units of activity (U) are expressed in micromoles of NADH per minute, and the enzyme concentrations are expressed as (U/L). The changes in absorbance are related to the following equation:

$$\frac{U}{L} = \Delta A/\min x \frac{2150\,\mu l}{50\mu l}\, x\, \frac{1}{6220}\, x10^6$$

$$\frac{U}{L} = 7235\; X\; \Delta A/min$$

Where 2150 = the total volume µl.

50 = the serum volume used.

6220 = molar extinction coefficient for NADH

10^6 = factor to convert concentration to µ mol/L.

Δ A = change in measured absorbance for time (t).

Determination of the serum superoxide dismutase activity: Superoxide dismutase (SOD) activity was estimated spectrophotrically by the modified by Weydert and Cullen in 2010 [7], the method is based on the ability of the enzyme to inhibit the reduction of nitroblue tetra-zolium (NBT) by superoxide generated during the reaction of photoreduction riboflavin and oxygen.

Procedures

1- 0.2 ml of EDTA/NaCN solution was added to 0.1 ml of the serum, and then 0.1 ml of NBT solution was added.

2- The assay tubes were brought to the standard temperature (20-22 °C), after that, 0.05 ml of riboflavin solution was added to each tube. The final assay volume of 3 ml was made up with phosphate buffer 0.067 M, pH (=7.8).

3- Subsequent tubes to bright lighting was controlled by placing the assay tubes in a white-light box where they received uniform illumination for 20 minutes with 18 w fluorescent tube attached to the lid, then the absorbance was read at 560 nm against distilled water.

4- To determine the control value, the absorbance for another set of tubes containing the same mixture was read at a 560 nm against distilled water, immediately after the addition of riboflavin which was added after the addition of the buffer solution.

5- To determine SOD unit, ten tubes containing (10, 20, 40, 60, 80, 100, 200, 300, 400, and 500 µL) of normal serum samples, and another tube containing no serum were treated as described in the above steps (steps 1, 2, and 3).

Calculations: Inhibition concentration was calculated from each absorbance using the following equation:

$$inhibition\,\% = (A_{NE} - A_E)\; X\; 100$$

Where:

A_E: The absorbance at 560 nm of the tubes containing different amount of the enzyme

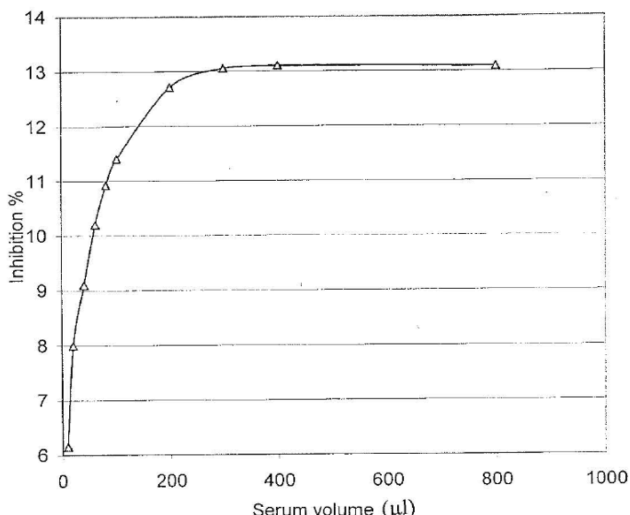
A_{NE}: the absorbance at 560 nm in the absence of the enzyme.

The percentages of the inhibition were plotted against the corresponding amount of serum as in figure 1

SOD units were calculated from figure 1 according to the volume of the serum which gives half of the maximum inhibition of NBT reduction (1 unit = 10.1 µl).

To calculate the SOD activity in sera of patients, the difference between absorbance before and after the light inhibition were multiplied by the SOD unit.

Fig. 1: Absorbance against serum volume



Blood sample preparation: The leukemia blood samples were classified in to three groups (ALL, CLL, CML) and one normal group. (Blood samples 5 ml were obtained from individuals of all groups by vein puncture and sera of all groups separated). The sera of all groups were lyophilized at the Al-Kindy Drugs Company.

RESULTS AND DISCUSSIONS

Determination of LDH activity in the sera of the patients with leukemia, Hodgkin's and control: The individual value of LDH activity for normal healthy controls and patients with leukemia and Hodgkin's are showed in table 1.

LDH levels are elevated significantly in all group of leukemia, Hodgkin's patients compared to normal. All the five isozymes present in leukemia and Hodgkin's patients agree with earlier reports [(8)].

It is observed in the present study that total LDH activity is found to be increased in patients who had no response during the course of chemotherapy this couldn't be useful in monitoring the response for treatment.

Elbossaty in 2017, found that LDH activity was significantly increased in all groups of leukemic patients, while those patients who successfully responded to the therapy shows decreasing in total LDH activity [(3)].

It is concluded that the total LDH activity could be as a biochemical marker for early assessment of response to serve therapy[9].

Determination of superoxide dismutase activity of sera in patients with leukemia, Hodgkin's and controls: Superoxide dismutase was determined in sera of leukemia, Hodgkin's and controls in patients, the SOD activity was measured using Weydert and Cullen 2010 [(7)]. Table 2 shows the individual values of SOD activity, the results revealed that the SOD activity decreased in patients compared with normal individuals and controls, whereas a slight increase in SOD activity for Hodgkin's compared with leukemia patients who show higher level of LASA compared with normal healthy and controls individual. No statistically difference was found between all types of leukemia and Hodgkin's patients.

It has been reported that there is a relationship between SOD activity and cancer, Ito et al., 2002[10], has reported that a lower levels of SOD in various malignant humors compared with normal cell lines, and also Truong-Minh et al has found that high metastatic cell lines contain less SOD than low metastatic cells[11].

Tumor cells have been shown to produce superoxide free radicals, if the rate of production of superoxide ion in tumor mitochondria is comparable to that found in mitochondria from normal tissue, then the loss of SOD would result as a net increase in the level of superoxide ion in the tumor cel[11].

Differences in the serum activity of SOD in patients with cancer disease are probably due to the decreased enzyme level in the tumor cell. Another possibility is a decrease in the synthesis and release of SOD from the blood cells because of the malignant immune deficiency[12].

The values of serum SOD activity obtained from this assay were correlated with LASA concentrations in sera leukemia, Hodgkin's and control patients. The results disclosed that, there is a significant negative between the two parameters. Consequently, the ratio of LASA/SOD was calculated for all groups of patients. Table 3 shows the values of LASA/SOD ratio for all types of leukemia is estimated to be about three fold higher, also Hodgkin's was about two folds higher compared with normal healthy individuals.

The LASA/SOD ratio was (27.69) in ALL and (30.6) in CML, (27.17) in CLL, (36.18) in AML leukemia, and (21.71) in Hodgkin's, the ratio was significantly higher in leukemia and Hodgkin's than in normal ($P<0.001$).

Serum levels of SOD and LASA reflect the changes in the content of membrane glycolipids and cellular activity of SOD.

Some authors admit that these changes in the tumor cells are in closed connection; the membrane of the neoplastic cell has an altered lipid content and structure origination, which leads to decreased antioxidant protection [(13)].

The loss of cell differentiation leads to an increase of the cell glycolipids, on the other hand, a decrease in intercellular of SOD. It has been reported that such a negative correlation does not appears in children with non-cancer disease. This is in accordance with observation that changes in membrane glycolipids and cellular antioxidants occurs in malignant tissues. These findings suggest that SOD and LASA are tumor markers[14].

Determination of sera traces elements and the electrolytes: The individual values of trace and electrolytes for the leukemia, Hodgkin's and pathological controls patients were determined by using the atomic absorption spectrophotometry. Figure 2 shows the results that indicate an elevation in Cu, Fe level and Cu/Zn ratio in sera of leukemia patients, also there is an increasing in Cu levels of Hodgkin's patients.

On the other hand there is a reliable decreasing in Zn, the levels of ser of leukemic and Hodgkin's patients, compared to control individuals which indicate the relationship between the three parameters (Zn, Cu, and Cu/Zn). The Mg, Ca levels in sera of leukemia and Hodgkin's patients remain in normal levels, but 11% of leukemia had hypocalcaemia and 15% of them had hypocalcaemia, the result also shows the abnormal increasing in ferrous level in some patients with CML before the death.

Alkhateeb and Connor 2013, shows that there is an increasing in ferritin level of tumor tissue in 27% of breast cancer patients which may indicate the increasing in ell numbers and its effect on the growth of cancer cells in breast[15].

It was assumed that the decreases in Zn level is related to the migration of Zn to the liver, this transfusion of zinc may help against spreading of cancer[16].

The increasing of copper to zinc ratio in the serum of ovarian cancer patients demonstrates the relationship between those 2 elements which can be utilized as an indicator of the nature of the tumor or it can be used in diagnosis and as a response of cancer treatment[17].

IR Spectra of leukemic sera: Infrared spectroscopy has been used in clinical laboratories, the analysis of the serum and other fluids from healthy and patients with various diseases including cancer. The spectra shows stretching,

bending, and other vibration bands that related to different groups as shown in figure 3 which include the following[18]

1. A decreases in the frequency face about 10 cm^{-1} in ALL, CLL, and CML leukemia sera due to secondary amine in the other chain side of the proteins.
2. Increase in frequency of about 40-50 cm^{-1} in the bands of CLL, CML, and ALL due to N-H stretching of the secondary amide in the polyamide backbone in the proteins and the NH_3 of the amines salts in the side chain of the proteins.
3. strong bands at (3525-3250 cm-1) is due to (O-H) stretching vibration of the intermolecular hydrogen bonded of such as non-estrified cholesterol, the hydroxyl group of Ser and The residues in the protein molecules and the hydroxyl groups of the carbohydrate (glucose molecules). This band is also due to (H-H) stretching vibration of the primary amines in (Lys, Arg) residues, primary amide in (Asp, Clu) residues in the protein molecules.
4. strong shoulder at 3350 cm^{-1} in ALL, 3400 cm^{-1} in normal and 3500 cm^{-1} in CML which were due to the N-H stretching vibration of the hydrogen bonded secondary amide (N-H) in the polyamide backbones in the proteins molecules. This band is also due to the (H-H) stretching vibration of ammonium group (NH_3) of side chase and terminal amino groups in protein molecules.
5. Weak combination of overtone bands appears at (2000-1780 cm^{-1}) is due to the (C-C) stretching vibration of the aromatic ring in the side chain of the protein and other serum compounds.
6. Weak shoulder at 1720 cm-1 is due to the presence of C=O stretching vibration of the ester group of phosphotides (esterified cholesterol triacyl glyceride) and unioxid carboxyl groups in the phospholipid acids.
7. Strong band at (1640-1620 cm-1) is due to C=O stretching vibration of the amide band (amide I band) of the poly amide backbone of the proteins (C=C).

Figure 2: The mean of the individual values of some trace elements and electrolytes in the sera of leukemia, Hodgkin's patients and control

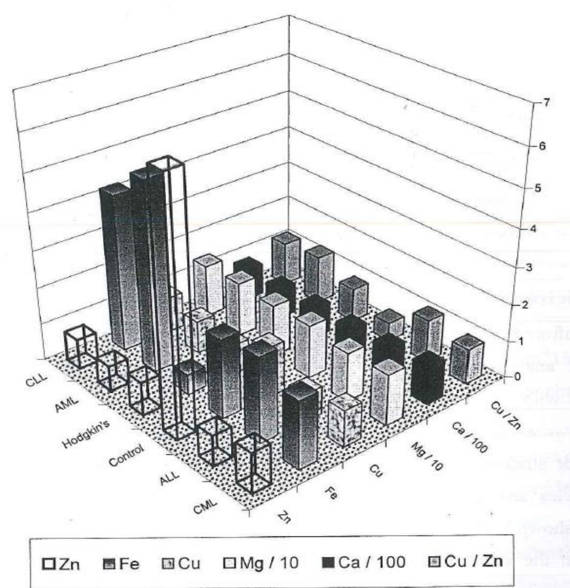


Figure 3: Infrared spectra of leukemic sera (ALL, CML, and CLL) and sera of normal individuals

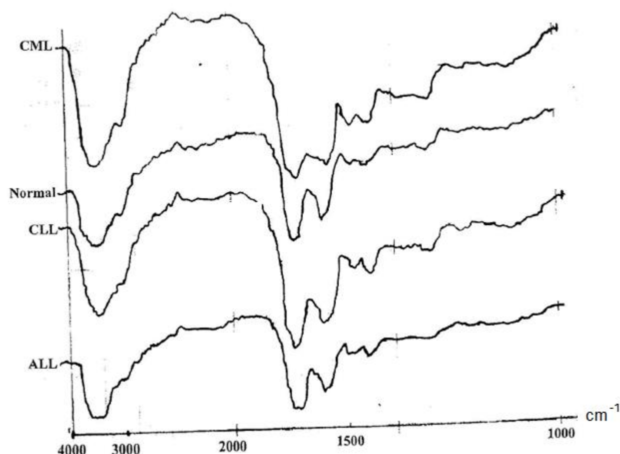


Table 1: Serum LDH activity with leukemia and Hodgkin's disease

Group	N	Mean U/L	±SD	T-Value	P=Value
ALL	16	367	144.2	7.43	0.0000
CML	15	314.7	111.1	7.48	0.0000
CLL	10	353.7	127	6.32	0.0001
AML	5	347.4	128.3	4.31	0.0013
Hodgkin's	12	294	119.7	5.38	0.0002

Table 2: Serum SOD activity in patients with leukemia and Hodgkin's disease

Group	N	Mean	±SD	T-Value	P-Value
ALL	12	1.4017	.1073	-8.02	0.0000
CML	8	1.3800	.1166	-6.55	0.0003
CLL	7	1.3086	.0979	-9.23	0.0001
AML	5	1.2480	.0642	-14.00	0.0002
Hodgkin's	8	1.4375	.1316	-4.57	0.0027
Control	7	1.8280	.2241	1.77	0.1300
Normal	6	2.0100	.2300	---	---

Table 3: Serum SOD activity, LASA and LASA/SOD ratio in patients with leukemia and Hodgkin's disease.

Group	SOD activity		LASA mg/dl		LASA/SOD	
	Mean	± SD	Mean	± SD	Mean	± SD
ALL	1.401667	0.107266	38.58333	7.089792	27.69583	7.171079
CML	1.380000	0.116619	40.50000	12.27076	30.61625	11.42698
CLL	1.308571	0.097882	35.00000	7.071068	27.17571	7.453021
AML	1.248000	0.064187	44.60000	13.16435	36.18000	12.47546
Hodgkin's	1.437500	0.131557	30.37500	10.59565	21.71875	9.695661
Control	1.828750	0.222418	28.62500	6.926914	15.27500	5.139969

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