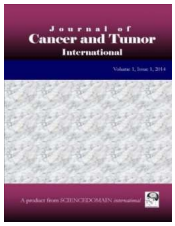

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Assessment of Survivin and Lactate Dehydrogenase Isoenzyme in Leukemic Egyptian Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author FTA designed the study, wrote the protocol, managed the literature searches, performed the analyses of the study author EMAEA designed the study, wrote the protocol and wrote most of the manuscript and author MHY managed the experimental process and author AASED was the clinical supervisor and identify the studied patient groups. All authors read and approved the final manuscript.

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ABSTRACT

Accurate diagnosis and proper monitoring of cancer patients remain important obstacles for successful cancer treatment. The search for cancer biomarkers is carried out in order to quickly identify tumor cells and predict treatment response, ultimately leading to a favorable therapeutic outcome.

The goal of this study is to highlight the importance of survivin level in cases of acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) to find its possible role in the pathophysiological development of the disease. Also to assess the oxidative stress in leukemic patients and to elucidate the relationship between erythrocytes lactate dehydrogenase (LDH) isoenzymes, antioxidant status, and survivin protein expression. This was achieved by detection the survivin-positive lymphocyte, erythrocyte glutathione peroxidase (E-GP-x), both of vitamin E and C,

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erythrocyte and serum LDH isoenzymes levels, and malondialdehyde (MDA). This study included 40 patients divided into two groups: 20 patients with CLL and 20 patients with ALL, together with 20 healthy control subjects (two groups age matched). The study showed a significant elevation in serum survivin protein, total LDH and MDA levels in patients with acute and chronic leukemia in comparison to healthy subjects. In contrast, vitamin C levels were decreased significantly in both studied groups. Strong correlation between survivin-positive lymphocyte and MDA was evident in ALL and CLL patients in the other hand a significant negative correlation between survivin and vitamin C in ALL patients was observed. A highly significant increase in serum levels of LDH isoenzymes commonly recorded in ALL patients with significant increase in LDH 1, LDH3, LDH4, and LDH5 in CLL group. Also erythrocytes LDH 4 and LDH5 show a highly significant increase and highly significant decrease in LDH2 in both studied groups. These data suggest that survive may be associated with the development of acute and chronic leukemia.

Keywords: Leukemia; surviving; oxidative stress and LDH.

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) are the most common types of leukemia in children and in adults, respectively. ALL is a rare disease with an incidence of 1.2–1.4 per 100,000 populations per year in Europe. It is an aggressive malignancy, characterized by a sudden onset and rapid progression, and diagnosis usually requires urgent medical attention [1]. ALL patients suffer from refractory or recurrent disease and cannot be brought around with conventional chemotherapy. Treatment of acute lymphoblastic leukemia in adults presents a formidable challenge. While overall results have improved over the past 3 decades the long-term 10 year survival for patients aged less than 60 years is only in the range of 30–40% [2].

According to blood cancer society, chronic lymphocytic leukemia (CLL) is the result of an overproduction of abnormal lymphocytes, a type of white blood cell. Leukemia cells, which are produced in the bone marrow, then spread to other parts of the body through the blood stream. CLL usually develops in older adults, not children [3]. There are two types of CLL: Slow-growing and fast-growing. Slow-growing CLL may not require treatment for years. Fast-growing CLL is much more serious and demands aggressive treatment.

Historically, CLL has been considered as an accumulative disease of lymphocytes defective in apoptosis, and this particular mechanism, but not increased proliferation, was thought to contribute to leukemogenesis [4].

Programmed cell death is a feature of living cells, and damaged cells are eliminated in this way. Inhibitors of programmed cell death apparently prolong cell viability, so contributing to the occurrence and growth of tumors. It plays an important role in both carcinogenesis and cancer treatment. Regulation of apoptosis is balanced by signalling pathways between apoptosis-promoting factors, and anti-apoptotic factors [5-7].

The inhibitors of apoptosis proteins (IAPs) are a family of proteins that suppress both mitochondrial and death receptor mediated apoptosis primarily by inhibiting caspases. Currently, eight IAP members have been identified in humans. Among them, survivin is the most potent cellular caspase inhibitor [8].

Survivin (16 KD protein) is a cancer-related molecule, usually detected in the cytoplasm of tumor cells. It plays an important role in regulating both apoptosis and cell division. It is undetectable in normal tissues but highly expressed in most forms of human cancer and functions as both an inhibitor of cell death and a mitotic regulator. Overexpression of survivin reduces the sensitivity of neoplastic cells to death stimuli induced by gamma radiation or chemotherapeutic agents. Also, it is associated with increased risk of recurrence and poor outcome in a variety of cancers, including hematologic malignancies [9,10].

It functions in the cell cycle as an essential mitotic regulator being a member of the chromosomal passenger complex. Also cytoplasmic survivin is predominantly involved in preventing apoptosis [11].

The role of survivin in apoptosis inhibition has been the subject of controversy. Initially, survivin and other IAPs were postulated to selectively bind to and promote the degradation of active caspases [7].

Increased generation of reactive oxygen species (ROS) and an altered redox status have long been observed in cancer cells, and different studies suggest that this biochemical property of cancer cells can be exploited for therapeutic benefits. The cancer cells in advanced stage tumors frequently exhibit multiple genetic alterations and high oxidative stress [12].

Excessive amounts of ROS can cause irreversible oxidative damage to lipids, proteins, and DNA, which lead to cell death. It still remains unclear whether the production of ROS in apoptosis is an essential component of the apoptotic pathway primarily by inducing mitochondrial damage, or an epiphenomenon of mitochondrial dysfunction that is unrelated to the key signalling events leading to apoptotic cell death [13,14].

This study was conducted to evaluate the survivin as anti-apoptotic marker, antioxidant status and LDH isoenzymes in acute and chronic lymphocytic leukemia patients as a dependable tool in the pathophysiology and prognosis of the disease.

2. PATIENTS AND METHODS

This study included 40 patients with acute and chronic leukemia selected from those attending at the National Cancer Institute (NCI) Egypt, Cairo, and 20 apparently healthy normal volunteers.

Patient groups divided into two subgroups. Group I, Included 20 patients with de novo chronic lymphocytic leukemia with mean 51.3±14.1 years (8 females and 12 males). Group II, Included 20 child patients with de novo acute lymphocytic leukemia with mean 6.2±4.1 years (5 females and 15 males). Leukemia was diagnosed and classified according to laboratory tests used to diagnose; all patients had not been treated with therapeutic drugs before the study. The healthy subjects were divided into two groups: (C1) control group, 10 subjects with mean 47.9±15.3 years (4 females and 6 males). (C2) a second control group with mean 7.1±6.9 years (3 females and 7 males). C1 and C2 are related to group I and group II respectively. The study protocol was approved by Ain Shams University and National Cancer Institute (NCI) Committee.

2.1 Blood Specimens

Venous blood samples were collected from lymphocytic leukemia patients and healthy control in the morning after an overnight fasting. Each blood sample was divided into the following portions:

1. 0.5 ml lithium-heparinized blood was kept at 2 - 8℃ and assayed in the same day of the collection for erythrocyte glutathione peroxidase (E-GP-x).
2. 4.5 ml lithium-heparinized blood centrifuged immediately at 1000x g for 5 minutes and the plasma samples were separated rapidly. 0.25 ml of plasma was mixed with 1.0 ml of 6 g/dl meta-phosphoric acid (MPA), for stabilizing ascorbic acid. The plasma-MPA mixture was stored at -20℃ until assayed. The remaining of the plasma was divided into several aliquots, for the determination of malondialdehyde (MDA), and stored at -70℃ to be thawed only once for analysis.
3. 2 ml EDTA blood was kept at 2-8ºC and assayed within two hours of the collection for survivin, complete blood count (CBC) and LDH isoenzymes.
4. 2.0 ml clotted blood (tubes without additives) for the determination of biochemical investigations. Samples were left to clot at 37℃ for 20 minutes, and then centrifuged at 2000x g for 10 minutes. The serum was then separated and stored at -20℃ to be thawed only once for analysis of vitamin E, total lactate dehydrogenase enzyme (LDH).

2.2 Biochemical Assays

Whole blood, plasma or serum were subjected to the following investigation:

Detection of survivin in human blood lymphocytes by flow cytometry:

Samples were assayed for the presence of survivin using the Human Survivin Fluorescein-conjugated Antibody (IC6472F) flow cytometric kit (R&D Systems, Inc., Minneapolis, MN), according to the manufacturer's instructions. Human peripheral blood lymphocytes were stained with Mouse Anti-Human Survivin Fluorescein-conjugated Monoclonal Antibody or Isotype control antibody for a negative control (This isotype control has been derivatized with a quantity of fluorochrome that matched the F/P (fluorochrome/protein) ratio of R&D Systems monoclonal reagents). To facilitate intracellular staining, cells were fixed with flow cytometry fixation buffer (1% paraformaldehyde) and

permeabilized with flow cytometry permeabilization/ wash buffer. Flow cytometric analysis was generated a signal which could be detected in the FITC signal detector.

Serum total LDH was assayed colorimetrically according to Mathieu [15]. Serum and erythrocyte LDH Isoenzymes were assayed by gel electrophoresis according to the method of Opher et al. [16]. Peak heights of the electrophoretic pattern above the ruled baseline are measured in millimeters and, with the total LDH activity (U/L), enter into a programmable printing desk calculator to The calculate the absolute activity (U/L) of each isoenzyme.

Both serum vitamin E and vitamin C levels were determined according to Baker& Frank [17] and Roe &Kuether [18] respectively. Erythrocyte glutathione peroxidase (E-GP-x) concentration and lipid peroxidation product (malondialdehyde) were assayed according to Paglia and Valentine [19] and Draper and Hadley [20] respectively.

2.3 Statistical Analysis

Data was analyzed using SPSS win statistical package version 22. Numerical data were expressed as a mean ±standard deviation, and range.

For quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric test corresponding to student t-test for variables. Comparison between groups was done using non-parametric ANOVA test). *P* Values were two-tailed and were considered significant if < 0.05.

3. RESULTS

Data presented in Table 1. and Fig. 1. showed that, survivin expression was a highly significant increase (16.5 and 33.1%) in CLL and ALL groups respectively (P<0.001) in comparison to control groups. In addition, there was a highly significant difference between patient groups (P<0.001).

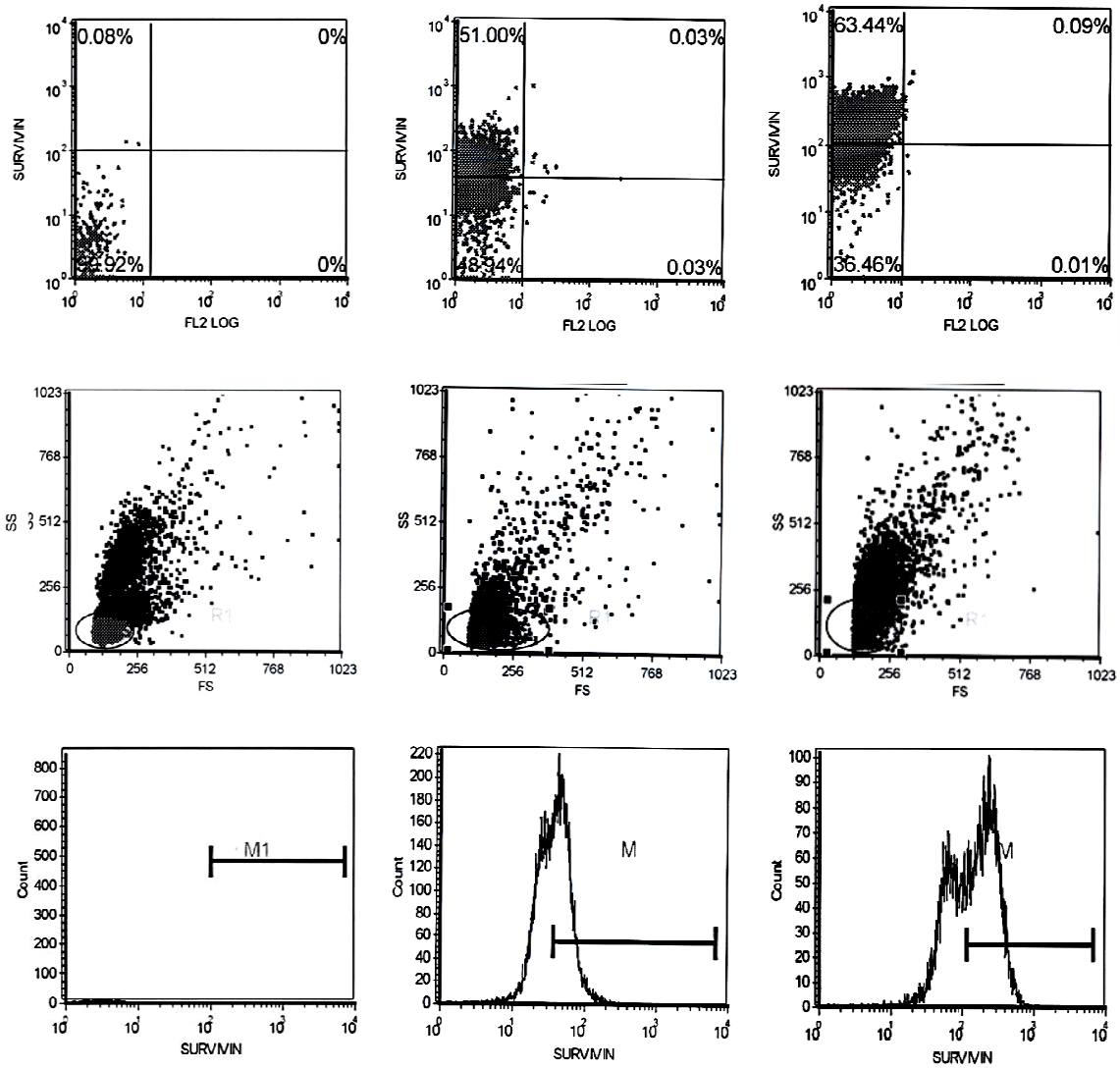
Serum T.LDH was significantly increased in CLL patients (P<0.05) and a highly significant increase in ALL patients (P<0.001) (159 and 435%, respectively) in comparison to control groups, with significant difference between patient groups (P<0.001) (Table 1).

WBCs and lymphocyte counts were a highly significant increase in CLL (322 and 996% respectively). While, in ALL group, WBCs and lymphoblast counts were significantly increased (325 and 455%, respectively) in comparison to the control group. In addition, there was no significant difference between patient groups.

Table 1. Survivin expression, serum T. LDH levels, lymphocyte and WBC's count in patients and control groups

Parameters / Groups	Survivin* (%)	T.LDH (U/L)	Lymphocytes (CLL) Lymphoblast (ALL)	WBC's
(C1) control				
Mean ± SD	0.3± 0.06	330±25.1	2.4±0.07	7.1±1.9
(C2) control				
Mean ± SD	0.3 ± 0.05	327.5 ± 13.3	2.9± 0.11	6.3±2.2
CLL Group				
Mean ± SD	16.8 ±2.2	855.5 ±74.9	25.67± 9.45	30.02±1 0.2
% Change	16.5↑	159↑	↑996	322↑
P<	0.001	0.05	0.001	0.01
ALL Group				
Mean ± SD	33.4 ±1.8	1757 ±142	16.1±2.33	26.8±11.6
% Change	33.1↑	435↑	↑455	325↑
P<	0.001	0.001	0.01	0.05
CLL vs ALL				
P<	0.001	0.001	N.S	NS

NS: non-significant (P>0.05); P<0.05: significant; P<0.001: highly significant.
**Represents percentage of survivin expression*



% of gated cells	Peak value
0.08	1.0

(a)

% of gated cells	Peak value
53.62	223.0

(b)

% of gated cells	Peak value
59.3	102.0

(c)

Fig. 1. Flow cytometry data analysis of positive survivin protein expression in CLL and ALL patient groups (b & c) in comparison to control groups (a)

FS: forward scatter. SS: side scatter

Results in Table 2. revealed that, plasma malondialdehyde was a highly significant increase in group-I and II (54 and 120%, respectively) (*P*<0.001) in comparison to control groups. A highly significant difference between patient groups was recorded (*P*<0.001). While, E.GP-x and serum vitamin E showed a non-significant difference either in CLL or ALL groups, but vitamin C was dramatically decreased (40 and 26%, respectively) (P<0.001) compared to control groups.

Fig. 2 and Tables (3 and 4), showed the electrophoretic distribution and absolute activities of plasma and erythrocytes LDH isoenzyme, where CLL group showed a significant elevation

in plasma LDH1, LDH3, LDH4 and LDH5 and highly significant increase in LDH2 (P<0.001). Also, our results revealed that a significant increase (P<0.05) in erythrocyte LDH1 and LDH4 with a highly significant elevation in LDH2 and LDH5.

Table 2. Antioxidants levels in CLL and ALL groups in comparison to the control group

Parameters / Groups	E-(GP-x) (U/g-Hb)	Vitamin C (mg/dl)	Vitamin E (µg/ml)	MDA (µM/L)
(C1) control				
Mean ± SD	14.3±1.7	1.7±0.38	13.7±1.1	3.1± 0.4
(C2) control				
Mean ± SD	16.2 ± 3.8	1.5 ± 0.3	13.2 ± 0.9	2.9 ± 0.6
CLL Group				
Mean ± SD	37.8 ±4.9	1.02 ±0.2	12.9 ±1.04	4.8 ±1.2
% Change	164.3↑	↓ 40	↓5.8	54.8↑
P<	NS	0.001	NS	0.001
All Group				
Mean ± SD	39.1 ±5.4	1.1 ±0.2	12.4 ±0.71	6.4 ±2.2
% Change	141↑	26↓	6↓	120↑
P<	NS	0.001	NS	0.001
CLL vs ALL				
P<	NS	NS	NS	0.01

NS: non-significant (P>0.05); P<0.05: significant; P<0.001: highly significant

Table 3. Serum LDH (total and isoenzymes) in control groups, CLL and ALL group

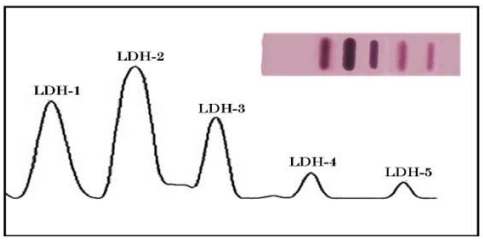
Parameter	T.LDH	LDH isoenzymes				
		1	2	3	4	5
		(U/L)				
Control (C1&C2)						
Mean± SD	329.5±13.3	93.3±3.4	132.3±4.1	66.5±2.1	20. 4±1.5	17.9±1.3
CLL Group						
Mean± SD	855.5±74.9	210.2±33.4	401.2±56.5	182.9±26. 7	25.7±4.8	20.9±2.6
P<	<0.05	<0.05	<0.01	<0.05	<0.05	<0.05
ALL Group						
Mean± SD	1757±142	435.7±111.8	848.2±183	400±98	28.1 ±6.6	22.1±4.8
P<	<0.001	<0.001	<0.001	<0.001	<0.01	<0.01
CLL vs. ALL						
P<	<0.001	<0.001	<0.001	<0.001	NS	NS

NS: non-significant; P<0.05: significant; P<0.001: highly significant

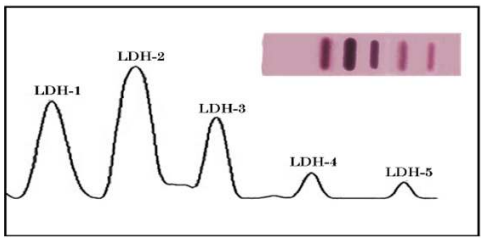
Table 4. Erythrocyte LDH (total and isoenzymes) in control groups, CLL and ALL groups

Parameter	T.LDH	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
			U/l			
Control (C1&C2)						
Mean± SD	98±21.1	31.4±0.7	45.9±3.1	15.9±0.5	4.7±0.6	1.9±0.2
CLL Group						
Mean± SD	112±15.3	38.0±7.4	35.1±2.02	16.5±2.3	5.1±1 .6	5.3±5.5
P<	N.S	<0.05	<0.01	NS	<0.05	<0.01
ALL Group						
Mean± SD	105±20.4	32.6±0.7	42.7±0.5	16.9±0.5	5.3±0. 8	4.6±0.3
P<	NS	NS	NS	NS	0.05	<0.01
CLL vs ALL						
P<		<0. 01	<0. 01	NS	NS	NS

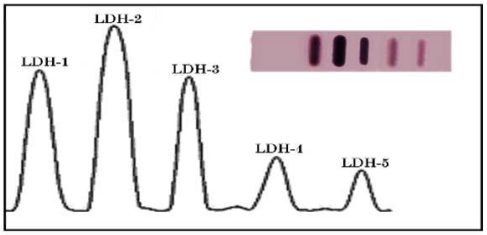
NS: non-significant; P<0.05: significant; P<0.001: highly significant



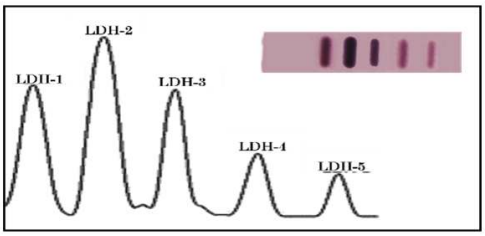
Electrophoretic separation of plasma LDH isoenzymes in control group C1



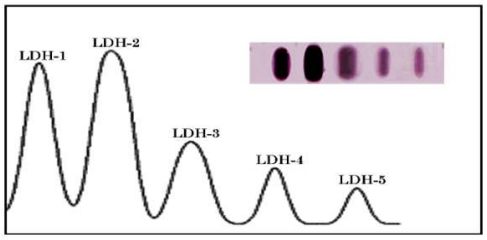
Electrophoretic separation of pasma LDH isoenzymes in control group C2



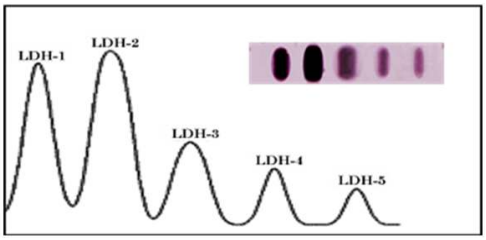
Electrophoretic separation of plasma LDH isoenzymes in ALL group



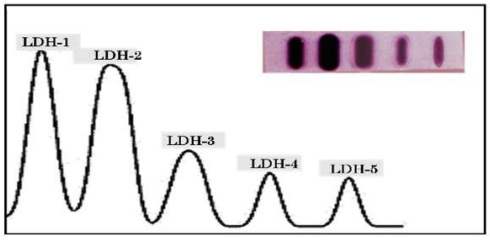
Electrophoretic separation of plasma LDH isoenzymes in CLL group



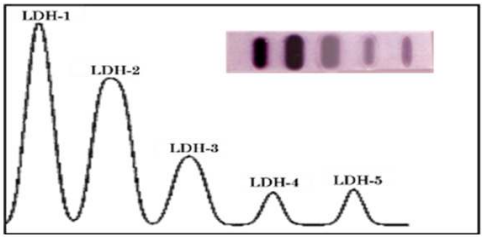
Electrophoretic separation of erythrocytes LDH isoenzymes in group C1



Electrophoretic separation of erythrocytes LDH isoenzymes in group C2



Electrophoretic separation of erythrocytes LDH isoenzymes in the ALL group



Electrophoretic separation of erythrocytes LDH isoenzymes in CLL group

Fig. 2. LDH isoenzyme distribution patterns of serum and erythrocytes in both control groups, ALL and CLL patients

In ALL group a highly significant increase in plasma LDH isoenzymes activities ($P<0.001$) were recorded. In the meantime, a highly significant increase ($P<0.01$) and a significant increase ($P<0.05$) in erythrocyte LDH5 and LDH4 respectively were recorded when compared to the control group.

Figs. (3-6) showed the correlation matrix of the different measured parameters and survivin, vitamin C and MDA for the different investigated groups of patients.

The data revealed that:

1. In CLL group: A significant positive correlation was shown between survivin expression, lymphocyte count and malondialdehyde.

2. In ALL group: A Significant positive correlation was shown between survivin expression and malondialdehyde. On the other hand, there were significant negative correlations between survivin expression and vitamin C.

4. DISCUSSION

American Cancer Society [21] has defined leukemia as the uncontrolled proliferation of hematopoietic cells that have lost the capacity to differentiate normally to mature blood cells. It usually invades the blood fairly quickly. They can then spread to other parts of the body, including the lymph nodes, liver, spleen, central nervous system and testicles (in males).

Survivin associated with increased risk of recurrence, loco regional lymph node invasion, and metastasis. In addition to its direct role in carcinogenesis, survivin may also play a key role in tumor angiogenesis because it is strongly expressed in endothelial cells during the remodeling and proliferative phase of angiogenesis [22].

Hence, it acts as a bifunctional protein through regulation of cell division and suppresses apoptosis. Anti-apoptotic properties of survivin play an important role in oncogenesis via direct or indirect inhibition of caspases. These properties would clinically provide a significant growth advantage and disclose malignant behavior in tumors [7].

The results of this study show a highly significant elevation in survivin expression in lymphocytes of ALL patients compared to control and a significant increase in CLL groups. Also, there was a highly significant increase of survivin expression in the ALL group when compared

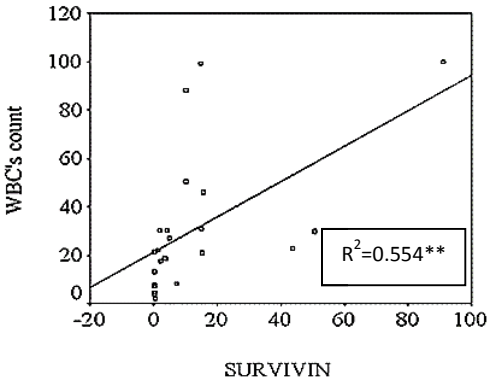


Fig. 3. Correlation between survivin expression and WBCs count in CLL group

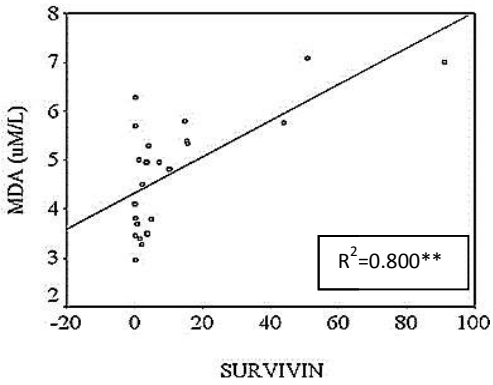


Fig. 4. Correlation between survivin expression and MAD in CLL group

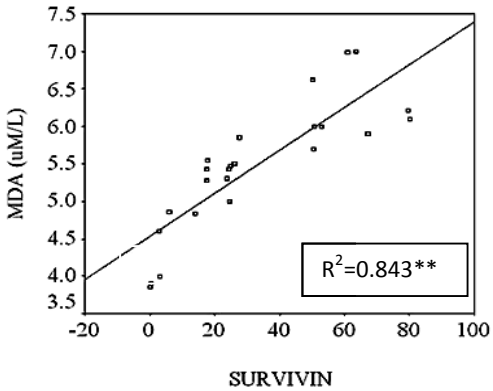


Fig. 5. Correlation between survivin expression and MDA in ALL group

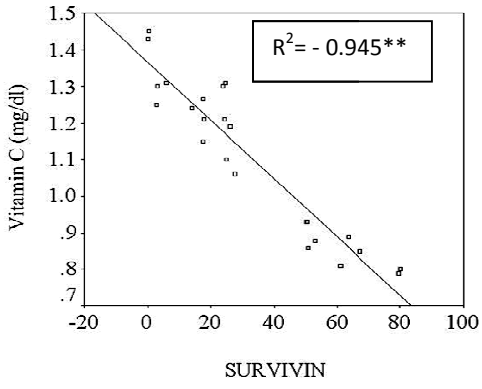


Fig. 6. Correlation between survivin expression and vitamin C in ALL group

with CLL group. These results are in agreement with Raida et al. [23] who reported that, over-expression of survivin was significant in CLL patients compared with the control group. In the meantime, Duffy et al. [24] also conclude that, survivin expression is up-regulated in approximately 90% of ALL cases. It is highly upregulated in most cancers. Particularly in lung cancer, expression levels of survivin appear to relate to disease prognosis [25].

Our results also were confirmed by Yao-Kuang et al. [26] who reported that the expression of survivin is present in most human neoplasm, but it is absent in normal and differentiated tissues. It is over expressed in the majority of lung cancer.

Also, Sun et al. [27] and Huang et al. [28] cleared that overexpression of surviving is closely related to the occurrence and development of acute leukemia, and may be used as an indicator of prognosis evaluation.

Data of the present study showed highly significant positive correlations between total leukocyte count and in CLL group and significant positive correlation with lymphoblast in the ALL group. These findings indicate that survivin has an important role in the pathogenesis and severity of the leukemia since the increase of lymphocyte in CLL and lymphoblast count in ALL is parallel with high survivin expression in lymphocyte cells which is responsible for prolonging the life span and the accumulation of these cells by enabling them to escape from their apoptotic fate.

Agata et al. [4] also declared that an increase in lymphocyte number is due to decreased apoptosis and slightly increased proliferation of B cells, this was observed in proliferation centers of pseudo follicles located in bone marrow, spleen, and lymph nodes.

Leukocyte count and lymphoblast level were found statistically increased in ALL patients compared to controls. This finding must be indicated presence the disease [29].

LDH plays a major role in hematological malignancies such as leukemia; lymphoma and myeloma that originate in the bone marrow or lymphatic tissues. As cells die, their LDH is released and finds its way into the blood. Hence, almost all hematological malignancies show elevated levels of serum LDH [30]. Also LDH, considered as a marker of anaerobic metabolism is associated with highly invasive and metastatic cancer [31].

The results of the current study revealed that, serum total LDH activity was a highly significant increase in ALL group and significantly increased in CLL group when compared to control groups.

A highly significant increase in LDH isoenzymes activities were recorded in ALL patients. Also, there is a significant increase in LDH 1, LDH3, LDH4, and LDH5 in CLL group. In the meantime erythrocytes LDH4 and LDH5 were a highly significant increase in ALL patients with significant elevations in LDH2 and LDH5 in CLL groups.

The previous variations in LDH isoenzyme activities encountered in different malignant neoplasms and the markedly elevated levels found in patients with ALL may reflect basic differences in cell proliferation and turnover in these disorders. In a condition such as ALL and CLL with rapid cell turnover, the serum LDH may raise, and the isoenzyme distribution usually reflects the site of the damaged organ and cells.

There is a good relationship between neoplasia and increased cellular LDH activity. It is moderately elevated in many cases of acute leukemia, irrespective to their cell type. Increased LDH reflects a shift towards anaerobic metabolism and increased glycolysis in the cytoplasm of malignant cells accompanied by a high turnover rate. Furthermore, tumors display glycolysis driven by hypoxia and/or oncogenic mutations, with the pyruvate to lactate conversion being promoted by increased expression of LDH [32]. The variations in the levels of serum LDH encountered in different malignant neoplasms and the markedly elevated levels found in patients with ALL may reflect basic differences in cell proliferation and turnover in these disorders [33].

Our data further showed that, a significant increase in the activities of both plasma and erythrocytes LDH 5 in ALL and CLL patients. It's the major LDH isoenzyme responsible for catalyzing the conversion of pyruvate to lactate and sustaining anaerobic glycolysis [34].

It can be inferred from these results that, the activities of T. LDH and its isoenzymes have been suggested to be valuable markers in identifying different kinds of malignancies,and

may be especially useful as adverse prognostic factors in hematological malignancies.

Reactive Oxygen Species (ROS) exert a key role affecting several hallmarks of cancer. ROS are involved in tumor angiogenesis, through the release of vascular endothelial growth factor and angiopoietin for evading apoptosis [35,36].

In cancer cells, high levels of ROS can result from increased basal metabolic activity, mitochondrial dysfunction due to hypoxia or mitophagy, peroxisome activity, uncontrolled growth factor of cytokines, signaling, and oncogene activity, as well as from enhanced activity of known ROS sources [37].

In the present study a significant increase in the plasma levels of MDA was observed in patients with ALL and CLL as compared to control.

Elevated level of lipid peroxidation products support the hypothesis that the cancer or malignant cells produce large numbers of ROS and that there exists a relationship between ROS activity and malignancy [38].

It is a well-known fact that activated oxygen species (AOS) are generated as a result of oxygen metabolism and energy production; they are also implicated in tumor progression [39].

In acute leukemia, increased ROS formation may introduce a signal transduction pathway to induce leukemic cells to proliferate but also lead to apoptosis. The serum contains a number of many different enzymatic and non-enzymatic antioxidants such as ascorbic acid, alpha tocopherol, beta carotene, uric acid, cortisol and albumin. Enzymatic antioxidants include Glutathione peroxidase, Glutathione reductase, Catalase and Superoxide dismutase (SOD) [40,41].

In this study, we have examined the activities of GP-x as enzymatic antioxidant and both vitamin E and C as non-enzymatic antioxidant defense system in ALL and CLL patients. A nonsignificant difference in GP-x activity was observed either in CLL or ALL groups in comparison to control groups or between the two patient groups.

With respect to Vitamin C levels in plasma, it was demonstrated that vitamin C was highly significantly decreased in groups I and II compared to control.

Also, our data recorded a non- significant change in vitamin E concentration in patient groups in comparison to control subjects. It is a membrane bounded antioxidant and can prevent the propagation of lipid peroxidation chain reaction by scavenging lipid peroxyl radical. It is described as anti-carcinogenic factor [40].

Our results are similar to those obtained by Mahmood et al. [42] who have shown a decrease in both vitamins. E and C with significant reduction in the activities of GP-x in ALL and AML patients.

The increase in free radical generation in leukemic patients and the decrease in antioxidant defenses are indicative of oxidative stress involved in the pathogenesis of human leukemia.

5. CONCLUSION

Survivin protein is related to anti-apoptotic proteins and has a dual role in both apoptosis and cell division, it is considered one of the important factors in the pathophysiology of leukemia. The present work provides evidence for the increased survivin expression in lymphocytes of CLL and lympho-blasts in ALL make it a reliable marker in the assessment and detection of leukemia. From our results, we concluded that the serum LDH and oxidative damage play an important role in the development of hematological malignancies. For confirmation of these findings, further studies on a larger sample size and long-term follow-up in patients are necessary to confirm whether these alterations are the cause or the consequence of carcinogenesis.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this paper.

ETHICAL APPROVAL

It is confirmed that all the authors have obtained all necessary ethical approval from ethical committee of National Cancer Institute (NCI).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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