



Prognostic significance of survivin and tumor necrosis factor-alpha in adult acute lymphoblastic leukemia

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ABSTRACT

Objectives: Acute lymphoblastic leukemia (ALL) is an aggressive cancer especially in adults as only 20–40% are cured with current treatment regimens.

Design and methods: We measured survivin and tumor necrosis factor-alpha (TNF- α) in serum of 30 ALL patients before and after induction therapy and compared to 30 age and sex matched normal adults.

Results: Survivin at cutoff value 15.18 pg/mL was detected in all ALL patients before therapy but in only 83.33% after therapy and not detected in the control group; $P < 0.001$. However TNF- α at cutoff value 60.05 pg/mL was detected in 90% ALL patients before therapy and 86.6% after therapy that was significantly higher than the control group (20%); $P < 0.001$. Survivin showed a significant positive correlation with TNF- α ($P < 0.05$), bone marrow blast cells ($P < 0.01$), peripheral blast cells ($P < 0.05$) and Philadelphia chromosome ($P < 0.01$).

Conclusions: Survivin may have an important role in the development of acute leukemia and it could serve as a significant prognostic marker.

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Introduction

Acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) are the most common types of leukemia in children and in adults, respectively. Some ALL patients suffer from refractory or recurrent disease and cannot be cured with conventional chemotherapy [1]. 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].

Tumor necrosis factor α (TNF- α) has a direct cytotoxic effect on various tumor cells. The most noticeable character of anti-tumor is specifically killing tumor cells in vivo and in vitro without toxic action on normal cells [3]. TNF- α is considered the most effective antitumor cytokine, it was found that its main mechanism is inducing apoptosis of tumor cells [4].

On the other hand survivin, a unique member of the inhibitor of apoptosis protein (IAP) family; plays an important role in regulating both apoptosis and cell division [5]. Overexpression of survivin is associated with increased risk of recurrence and poor outcome in a variety of cancers [6] including hematologic malignancies [7]. Expression of survivin is up to ten-fold higher in ALL blasts than in normal peripheral blood and bone marrow. The efficacy of survivin-specific cytotoxic T

cells has already been shown for both ALL as well as CLL [8]. In cancer patients, the fact that survivin overexpression may provide a survival benefit for tumor cells and that its enhanced expression is almost completely restricted to malignant tissues makes survivin an interesting target for the development of immunotherapeutic strategies [9]. Rödel et al. [10] reported that survivin is an antiapoptotic gene which is overexpressed in most human tumors and involved in mitotic checkpoint control. High levels of survivin expression have been associated with cancer progression, drug resistance, poor prognosis, and short patient survival. Interestingly Schmidt et al. [9] found that survivin expression can be up-regulated in activated B and T lymphocytes on stimulation with tumor necrosis factor α (TNF- α).

So far, there was no report about the relation of survivin protein in response to the therapy in acute lymphoblastic adult patients with poor survival outcome. The current study was therefore performed to determine survivin in serum and other apoptotic factor as TNF- α especially in adult ALL and assess their prognostic relevance.

Patients and methods

This study included 30 subjects selected from those treated at the Clinical Hematology Unit in Ain Shams University hospital in the period from 2008 to 2009 and 30 apparently healthy normal volunteers matching the patients for age and sex. Leukemia was diagnosed and classified according to the criteria of the French-American-British (FAB) Cooperative Group [11]. The subjects were divided into 3 groups: Control Group (Group A) included 30 normal adults (22 males and

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8 females) with mean 41.3 ± 11.1 years. Thirty acute lymphoblastic patients (21 males and 9 females) with mean 37.9 ± 14.3 years; they were further divided into: patients before receiving any treatment (group B) and follow-up patients after therapy (group C). They included 19 patients diagnosed as having pre B-ALL (2 with +ve Philadelphia chromosome), 2 patients as having B-ALL and 9 patients as having T-ALL (3 with +ve Philadelphia chromosome). The median percentage of leukemic blasts in the bone marrow and blood samples was 87% (range 50–97%). All bone marrow samples and the majority of peripheral blood samples contained more than 70% blast (range from 50 to 80%). The patients were treated with standard induction therapy of ALL [Adriamycin $25\text{mg}/\text{m}^2$ and vincristin $1.4\text{mg}/\text{m}^2$ days 1,8,15 and 22 together with L-Asparaginase $5000\text{ IU}/\text{m}^2$ for 14 consecutive days plus prednisolone $1\text{ mg}/\text{kg}/\text{day}$ for 28 days]. Patients with +ve Philadelphia, additionally received Imatinib $400\text{ mg}/\text{day}$, and were followed up by PCR for the expression of p210 fusion protein (BCR/Abl) to shift them to another treatment protocol (Hypper- CVAD protocol) [12]. Fortunately, Philadelphia +ve patients in this study did not express p210 fusion protein. All involved patients have shown good response to treatment proved by negligible bone marrow and peripheral blast cells. All patients were subjected to detailed history taking and medical examination concerning their disease. An informed consent was obtained from all included subjects.

Sample collection

Blood samples (5 to 10 ml) were drawn from all subjects into tubes without anticoagulant before any therapeutic measures were started and after 4 weeks of induction therapy. Serum samples were separated then stored at -80°C until subsequent processing and measurements.

Assay procedures

Survivin concentration was measured using enzyme-linked immunosorbent assay (ELISA) kit from Quantikine® Human Survivin Immunoassay (R&D Systems, Inc., Minneapolis, USA).

TNF- α concentration was measured using enzyme-linked immunosorbent assay (ELISA) kit from Quantikine® Human Tumor Necrosis Factor- α (hTNF- α) Immunoassay (R&D Systems, Oxon, UK).

The assay employs the quantitative sandwich enzyme immunoassay technique. The steps of both kits were those described by the manufacturer.

Statistical analysis

Data were analyzed using SPSS 15 software. Quantitative variables were reported as mean \pm SD. Student's *t* test was performed to assess whether the results are significant or not. The level of significance was set at $P < 0.05$. The associations between studied parameters and clinicopathological parameters were assessed by χ^2 test. The best cutoff value that maximizes sensitivity and specificity and differentiates acute lymphoblastic patients from controls was calculated by using the ROC (receiver operating characteristic) curve.

Results

The mean serum survivin level was significantly higher in acute lymphoblastic patients ($28.00 \pm 11.23\text{ pg}/\text{mL}$; range $11.6\text{--}63.6\text{ pg}/\text{mL}$) than in the control group ($9.05 \pm 3.02\text{ pg}/\text{mL}$; range $3.31\text{--}14.77\text{ pg}/\text{mL}$), $P = 0.00$. A significant reduction of survivin level was observed in ALL patients after therapy (mean $20.7 \pm 5.6\text{ pg}/\text{mL}$; range $11.6\text{--}30.5\text{ pg}/\text{mL}$) than in ALL patients before therapy (mean $35.28 \pm 10.71\text{ pg}/\text{mL}$; range $20.5\text{--}63.6\text{ pg}/\text{mL}$), as shown in Fig. 1. TNF- α was also significantly higher in acute lymphoblastic patients (mean $231.98 \pm 116.5\text{ pg}/\text{mL}$; range $50.0\text{--}476.4\text{ pg}/\text{mL}$) than in the control group (mean $51.9 \pm 41.25\text{ pg}/\text{mL}$; range $20.53\text{--}190.74\text{ pg}/\text{mL}$), $P = 0.00$. TNF- α showed

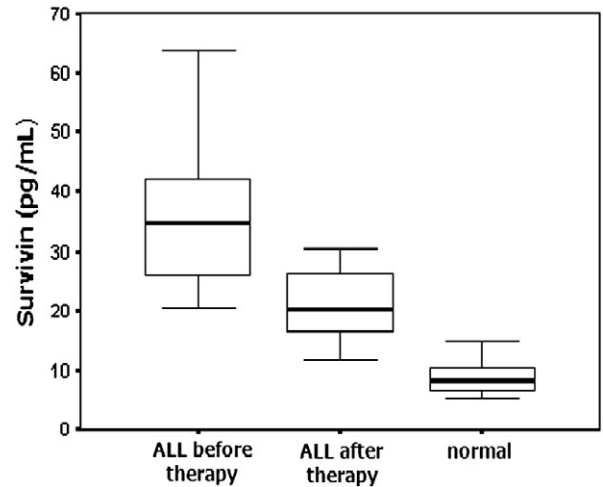


Fig. 1. Serum survivin level in ALL patients before and after therapy and control groups.

significant lower levels in ALL patients after therapy (mean $180.84 \pm 98\text{ pg}/\text{mL}$; range $44.47\text{--}384.47\text{ pg}/\text{mL}$) than before therapy (mean $275.13 \pm 108.3\text{ pg}/\text{mL}$; range $50.0\text{--}476.4\text{ pg}/\text{mL}$) as shown in Fig. 2. Serum survivin showed a higher significant reduction in its level after therapy than TNF- α ; ($P < 0.01$) and ($P < 0.05$) respectively. The best cut-off values for investigated parameters were calculated by the ROC curve as $15.18\text{ pg}/\text{mL}$ for survivin and $60.05\text{ pg}/\text{mL}$ for TNF- α (Fig. 3). On the contrary to TNF- α , the percentage of patients with survivin concentrations above the cutoff value was significantly higher in ALL patients before therapy than after therapy ($P < 0.05$), while no significant reduction was found with TNF- α . (Figs. 4 and 5). Moreover, survivin showed a positive significant correlation with TNF- α ($P < 0.05$), bone marrow blast cells ($P < 0.01$) and blast cell count in peripheral blood ($P < 0.05$), but TNF- α was only correlated with blast cell count in peripheral blood ($P < 0.05$). As regards Philadelphia chromosome, it was positively correlated to both peripheral and bone marrow blast cells, Furthermore correlated to survivin ($P < 0.01$) and TNF- α ($P < 0.05$).

Discussion

Acute leukemia is the 4th most common cancer among males and the 3rd most common cancer among females [13].

Impaired apoptosis is mediated by members of the inhibitor of apoptosis proteins (IAP) family such as survivin. Survivin was described in a number of different tumors and found to correlate with poor prognosis in a variety of cancers including hematologic malignancies

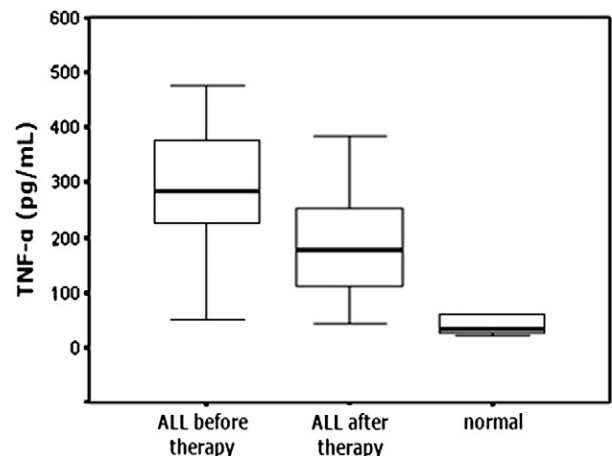


Fig. 2. Serum TNF- α level in ALL patients before and after therapy and control groups.

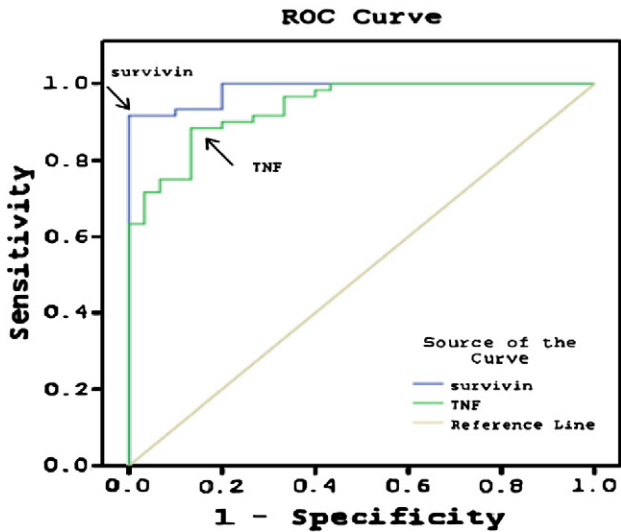


Fig. 3. Receiver operating characteristic (ROC) curves for serum survivin and TNF- α to discriminate between ALL patients and normal control group. The arrows denote best cut-off points of survivin at 15.18 pg/mL (sensitivity = 91.7%, specificity = 100%, area under the curve (AUC) = 0.985, $P < 0.01$) and TNF- α at 60.05 pg/mL (sensitivity = 90.0%, specificity = 80%, AUC = 0.94, $P < 0.01$).

[14]. It suppresses tumor cell apoptosis triggered by chemotherapeutic agents, caspase 3, 7, and 9, or Fas ligand-induced stimuli [15]. The role of TNF- α in ALL is very complex. The endogenous production of TNF- α in ALL blasts is connected with their resistance to chemotherapy. Moreover, a high serum level of TNF- α can be a predictor of early disease relapse [4]. It was proved that stimulation of venous smooth muscle cells in human with Angiotensin II and TNF- α led to a rapid up regulation of transcription factor, Kruppel-like factor 5, that increase survivin expression [16].

In the present study; survivin and TNF- α levels were estimated in the circulation of adult patients with newly diagnosed untreated ALL and after initial treatment in order to find out their implication in the pathogenesis of ALL and to evaluate their prognostic value. We used a

methodologically simple ELISA that can be applied to large clinical studies. The results demonstrated significantly increased levels of survivin and TNF- α in acute lymphoblastic patients compared to normal control group, suggesting that both survivin and TNF- α have relevant biological activity in ALL patients. Altieri [17] reported that survivin overexpression has been identified as a negative prognostic factor in various cancer types and was implicated in resistance to apoptosis induction by anti-cancer agents. Also, Drabko et al. [18] found that serum TNF- α level was increased in children with ALL compared to control group. Our results are supported by other authors, but so far; the prognostic value of these two biochemical markers in adult leukemic patients was not previously elucidated. In the present study a significant reduction in both survivin ($P = 0.00$) and TNF- α ($P < 0.05$) in acute lymphoblastic patients was clearly detected in response to the treatment. On contrary to TNF- α that showed no significant difference in its positivity rate in response to therapy, survivin had a significant higher positivity rate in acute lymphoblastic patients before therapy (100%) in comparison to that after therapy (83.33%). Esh et al. [14] published that pediatric ALL patients who were suffering relapse of the disease had a significant higher basal level of survivin detected by flow cytometry than patients still in remission. Although survivin and TNF- α protein in this study were reduced in response to therapy, their levels were still higher than in the control group. This may probably be due to short follow-up period (4 weeks) of the patients or it may be due to the lysis of circulating tumor cells and the release of their intracellular survivin or it could indicate the persistence of residual tumor cells. This may explain the finding of Guney et al. [19] that serum survivin levels did not change significantly after chemotherapy in patients with breast cancer. Moreover, Potapnev et al. [20] detected higher plasma levels of TNF- α in childhood ALL compared to healthy children that showed no significant association with treatment response.

Patients with acute- or lymphoma-type adult T-cell leukemia (ATL) have a poor outcome because of the intrinsic drug resistance to chemotherapy. Protection from apoptosis is a common feature involved in multidrug-resistance of ATL [21]. However, we couldn't find any significant correlation between the microscopic types of ALL with survivin or TNF- α .

The observation that inhibition of survivin interactions by antisense or dominant-negative approaches in different transformed cell models

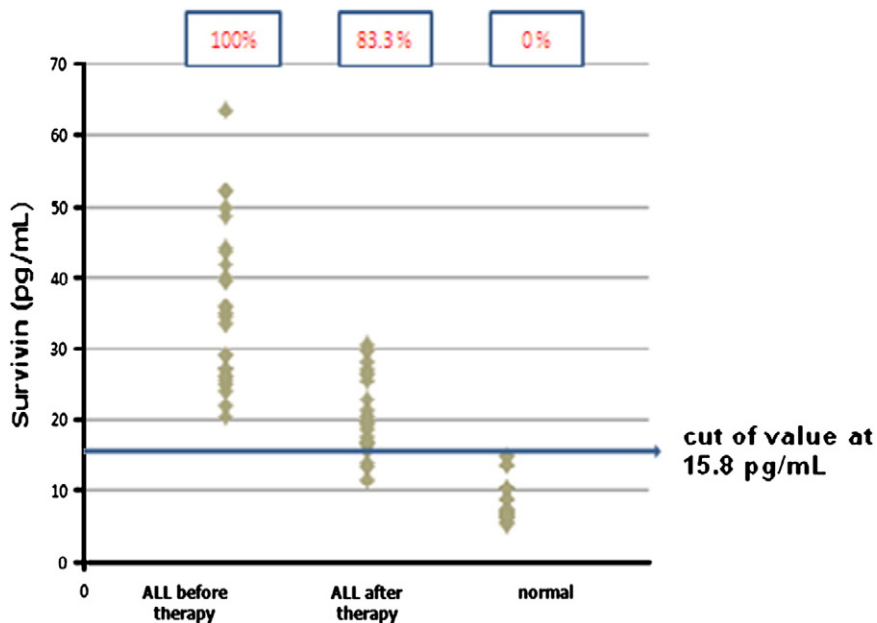


Fig. 4. The percentage of patients with serum survivin above the cutoff value in the different studied groups.

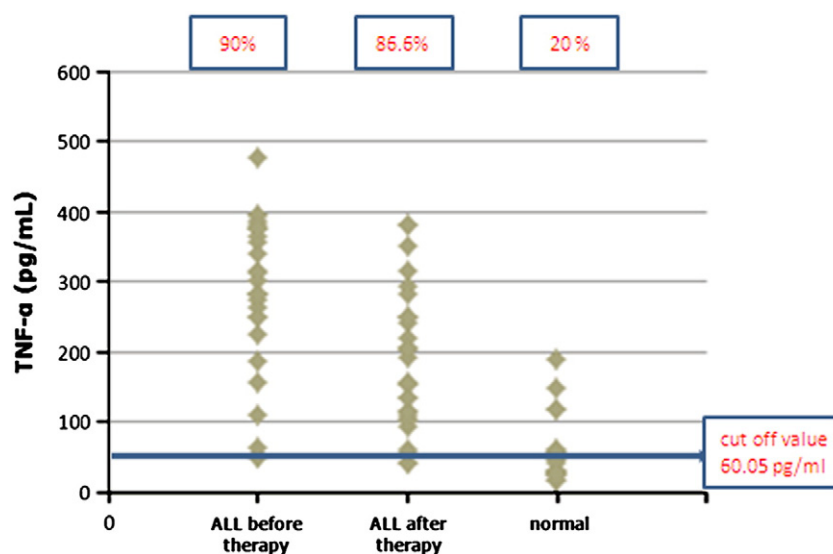


Fig. 5. The percentage of patients with serum TNF- α above the cutoff value in the different studied groups.

can induce apoptosis of malignant cells led to the assumption that targeting of survivin could represent a novel strategy to treat cancer patients [9]. In the current study, survivin showed a positive significant correlation with both bone marrow blast cells and blast cell count in peripheral blood, while TNF- α was only correlated with peripheral blast cells. This was agreed with Potapnev et al. [20] who demonstrated a positive correlation between blast cell count in peripheral blood and TNF- α . However, Esh et al. [14] reported no association of survivin levels with established risk factors.

Malcles et al. [5] showed that survivin- Δ Ex3, one of the spliced variants, is a central regulator at the mitochondrial checkpoint during TNF- α induced apoptosis, where it binds to Bcl-2 and to activated caspase-3, acting as an adaptor, which allows Bcl-2 to inhibit the activity of caspase-3. This may explain the positive correlation between survivin and TNF- α detected in the present study and demonstrate the antiapoptotic effects of survivin through opposing the apoptotic effect of TNF- α and survival of malignant cells.

The characteristic large change in the chromosomes of cancer cells is an important predictor of outcome. Some cytogenetic subtypes have a worse prognosis than others [21]. Paietta et al. [22] reported that a translocation between chromosomes 9 and 22, known as the Philadelphia chromosome, occurs in about 20% of adult cases of ALL. In this study 16.6% of our ALL patients has Philadelphia chromosome. They were positively correlated to both peripheral and bone marrow blast cells that was agreed with their bad prognostic factor in ALL patients. Moreover, our positive Philadelphia chromosome patients showed a higher significant positive correlation with survivin ($P < 0.01$) than with TNF- α ($P < 0.05$).

These results indicated that serum survivin and TNF- α levels are increased in patients having ALL and they correlated with known established risk factors in ALL as peripheral blast cells and Philadelphia chromosome. However, survivin showed more significant correlation with bone marrow blast cells and other prognostic criteria in ALL, suggesting that survivin may enhance the aggressive behavior of this tumor. As compared with TNF- α , survivin had a better sensitivity and specificity cut off value that showed a significant lower positivity rate in ALL patients in response to the therapy. We conclude that the survivin serum level correlates with the extent of disease and associated with aggressive clinical behavior, highlighting the usefulness of survivin as a potential diagnostic and a novel prognostic factor for survival in ALL. Future studies should focus on the prospective analysis of these assays against long follow up treatment in larger multicentric studies for better management of ALL patients.

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