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EXPRESSION OF SOME APOPTOTIC INHIBITORS IN CASES OF ADULT ACUTE MYELOID LEUKEMIA

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ABSTRACT

The expression of antiapoptotic genes; bcl-2, Survivin and Aven was examined in Egyptian cases of acute myeloid leukemia (AML). Flow cytometry (FCM) was used to detect the expression of bcl-2 and reverse transcriptase-PCR was used to examine the expression of Survivin and Aven mRNA**.** Bcl-2 was highly expressed in all cases of AML than controls (p< 0.001). The percentage of bcl-2+ cells was significantly higher in M1 (60%), M2 (54%) and M3 (56%) than in M4 (43%) and M5 (40%) subtypes (p=0.01). The AML patients showed expression of Survivin mRNA but no expression of Aven mRNA was observed. Out of 30 patients, 23 (76.6%) showed detectable levels of Survivin expression. The ratio between Survivin mRNA and beta-Actin mRNA was analyzed quantitatively in AML samples showing expression bands. Different expression levels of Survivin mRNA could be observed between different FAB subgroups. M1, M4 and M5 AML had a relatively higher expression level of Survivin mRNA (range: 0.56-0.81) than M2 and M3 patients (range:0.04-0.1). There was also a significant correlation between Survivin expression and PB blast % in cases of M5 subtype of patients (*P*=0.02). The Survivin-positive AML cases showed a higher percentage of bcl-2 expression and a significantly higher Bcl-2 MFI (mean fluorescence index) in comparison with Survivin-negative ones (p= 0.01). Our findings suggest that over-expression of both apoptotic inhibitors Bcl-2 and Survivin might be involved in AML pathogenesis.

INTRODUCTION

Apoptosis is a morphologically distinct genetic program of cellular suicide which provides a vital protective mechanism against the development of neoplasia by removing cells with DNA damage. It also plays a central role to the homeostasis of adult tissues by maintaining the balance between cell production and cell elimination. Inhibition of apoptosis thus confers a survival advantage on cells harboring genetic alterations and may promote acquisition of further mutations to cause neoplastic progression and also contribute to the development of resistance to chemotherapy (1, 2).

A large number of evidences show that many molecules such as p53 and bcl-2 are involved in the regulation of apoptosis during tumorigenesis. BCL2 (B-cell lymphoma/leukemia-2) is an oncogene located on human chromosome 18q21 identified originally as a novel transcript associated with the t (14;18) chromosomal translocation which occurs in most follicular lymphomas. It encodes for a protein of 239 amino acids (Bcl2-alpha; 26kDa) that is located in the inner

mitochondrial membrane. Bcl2 has been localized also to other membranes, including the endoplasmic reticulum and nuclear membrane (3,4). The over-expression of BCL2 prevents death by apoptosis of B-cells and T-cells and counteracts apoptotic mechanisms elicited by cross-linking of the APO-1 cell surface protein (5,6).

The inhibitor of apoptosis proteins (IAPs), which are widely expressed in all kinds of malignancies are encoded by the highly-conservative anti-apoptosis gene family and play an important role in the regulation of apoptosis through caspase-dependent and caspase- independent mechanisms (7,8). In humans, several members of the IAP family have been described: HIAP1, HIAP2, XIAP, NIAP, *Survivin* and, more recently, *Livin* and Aven (9-11).

Survivin is a 16.5kDa protein with a single baculovirus inhibitor of apoptosis (IAP) repeat (BIR) domain (9). It has been mapped to chromosome 17q25 (12). Survivin is expressed during embryonic development but is absent in most normal, terminally differentiated tissues. *Survivin* has also been detected widely in a variety of human tumors, including breast, colon, pancreas and prostate carcinoma, neuroblastoma, melanoma and non- Hodgkin's lymphoma (9). Studies performed by immunohisto-chemistry described the presence of *Survivin* in a variable percentage of tumors, ranging from 30% of gastric cancers to 90% of melanomas (10, 13). Most data obtained from these studies suggest that survivin expression in cancer appears to be associated with unfavorable clinical and pathological parameters, such as poor prognosis with progressive diseases and shorter patient survival rates (14, 15).

Survivin plays a role in the proliferation and survival of normal hematopoietic cells. Survivin expression is aberrantly enhanced in most cancers and hematopoietic malignancies (16). Some hematological studies examined by immunocytochemistry the expression of survivin in bone marrow cells from acute myeloid leukemia (AML) cases and patients with myelodysplastic syndrome (MDS) and confirmed the high incidence of survivin expression in AML. They also suggested that survivin abnormal expression also in MDS may play a role in promoting aberrantly increased cell viability and contribute to the altered homeostatic balance between cell growth and cell death (17, 18). The expression of Survivin and its splicing variants was also examined by quantitative real-time RT-PCR method in patients with acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL), and has found to be differentially regulated in ALL and CLL cells. (19)

Another recently discovered anti-apoptotic 38.5KDa protein, Aven suppersses apoptosis induced by Apaf-1 and caspase-9 (20). Aven gene is located on chromosome 15q1. Aven expression has recently been examined in children with acute lymphoblastic leukemia (ALL) for possible correlation with clinical features at diagnosis and treatment outcome. It was found to be higher in patients with unfavorable cytogenetic abnormalities. Aven expression was also significantly higher in relapsed patients in the standard-risk group (21). Aven is less widely studied in cancer cells but its anti-apoptotic feature is known (20).

The aim of present study was to examine the expression of both *Survivin and Aven* messenger RNA (mRNA) as well as levels of Bcl-2 oncoprotein

expression in cases of acute myeloid Leukemias with different FAB subtypes and to evaluate any association between expression of this gene and relevant clinicopathological features.

MATERIALS AND METHODS

Sample collection: This study included a total of 30 patients with newly diagnosed Acute Myeloid Leukemia admitted to the hematology unit at the Main University hospital; Alexandria over the period from May 2006 - February 2007. The recruited patients were 14 men and 16 women; aged 24-58 years (mean (42±9) years). Ten healthy volunteers, with matched age and sex ratio, were included in the control group. Informed consents were obtained from all participants and the local Ethical Committee approved the use of blood for scientific studies.

All patients were diagnosed using routine procedures, including morphology, cytochemistry. In addition,immunephenotyping, for CD34, CD13, CD33, CD14, CD19, CD7, cCD3 were performed for all patients. Cases were classified according to the French-American-British (FAB) classification with respect to cell lineage and degree of differentiation. This classification is a lineage-based system that broadly divides AML (M0-M7) according to blast morphology as well as maturation and differentiation (22).

Detection of Bcl-2 oncoprotein using flowcytomery: The heparinised blood samples were layered on equal amounts of Ficoll hypaque for separation of MNCs (mononuclear cells). Bcl2- was analysed using FITC (fluorescein isothiocyannate) conjugated mouse antihuman Bcl-2 oncoprotein (23, 24)

Cell counts of $1x10^6$/ml MNCs were used per sample. The cells were fixed for 10 min at room temp with 2% paraformaldehyde in PBS then permeabilized using 0.5% Tween 20 in PBS. The fixed cells were incubated with 10µl of Mouse-Anti human bcl2 clone 124 (DAKO-bcl-2, 124) for 15 min at room temp after 2 washes with 0.5% Tween 20 as mentioned above. The cells were incubated with 10µl of FITC-conjugated second antibody (rabbit anti-mouse immunoglobulin) for 10 min at room temp in the dark. The cells were then washed twice with PBS and resuspended in 500µl of PBS and were ready for analysis using Galaxy Flow Cytometer supplied by DAKO, Denmark (on software: Flomax).

Isotypic antibodies were used as negative controls. In all cases 10,000 events were acquired and Detection was gated primarily on the subpopulation of malignant cells by forward and side scatter dual parameter.

Bcl-2 expression was defined by considering both the percentage of positive cells and the mean fluorescence index (MFI) expressed as the ratio of sample mean channel/control mean channel (25).

Detection of Survivin and Aven mRNA using RT–PCR

Messenger RNA extraction: Peripheral blood samples were taken from the patients (study group) and from healthy subjects (control group) into vacutainer EDTA tubes. White blood cell (WBC) counting was performed in Sysmex KX-21 cell counter. Red cells were removed by centrifugation after lyses with red blood cell lysis buffer. The WBCs were suspended with lysis buffer and mRNA isolations were performed using Trizol® reagent (Invitrogen).

Reverse transcription and complementary (c)DNA synthesis: RNA (0.1-0.5µg) was reverse transcribed using Revert Aid ™

first strand cDNA synthesis Kit (Fermentas) according to the manufacturer's guidelines. Briefly reverse transcription was carried in a 20 µl reaction containing 1µl Revert Aid M-MuLV Reverse Transcriptase enzyme, 10 mM of each dNTP, 1µl of random hexamer primer, and 1µl RiboLock Ribonuclease inhibitor. The reaction mixture was incubated at 25°C for 10 min, then at 42°C for one hour followed by incubation at 95°C for 10 minutes to stop the reaction. cDNA samples were stored at –20°C until RT PCR was performed.

Primers: Sequences of the primers used are as follows (26, 27):

β-actinF
5′-TTAGCTGTGCTCGCGCTACTCTCTC-3′
β-actinR
5′-TCGGATTGATGAAACCCA GACACA 3′
Survivin SF,
5′-CAGATTTGAATCGCG GGACCC-3′
Survivin SR,
5′-CCAAGTCTGGCTCGTTCTCAG-3′

the survivin mRNA, GenBank accession number is NM 001168

Aven AF
5′-GATTTCAGTGTCCTCCT TAG- 3′
Aven AR
5′-CCTTGCCATCATCAGTT CTC-3′.

The aven mRNA Genbank accession number is AF283508.

Detection of mRNA expressions using PCR: Aliquots of the cDNA were amplified with Aven and Survivin primers. PCR reaction was performed in a final volume of 20µl containing 1µl cDNA, 0.2 µM of each oligonucleotide primer, and 10µl of GoTaq® Green master mix, (Promega, Madison, WI, USA) containing 400µM dNTPs, 3mM magnesium chloride and reaction buffer (pH 8.5). Thirty cycles of PCR amplification were performed in a DNA thermal cycler with denaturing at 94°C for 30s, annealing at 60°C for 30s (54+0.2°C for Aven) and extension at 72°C for 30s. PCR products were visualized on 3% agarose gels with ethidium bromide staining under ultraviolet transillumination. A sample with out RNA was included in each RT–PCR as a negative control.

To ensure the fidelity of mRNA extraction and reverse transcription, all samples were subjected to PCR co-amplification with oligonucleotide primers specific for the constitutively expressed gene β-Actin.

Quantitative analysis of RT-PCR Products: RT-PCR products were electro-phoresed through 3.0% agarose gel, stained with ethidium bromide and visualized using UV transilluminator. We used the image analysis software program **Totallab** to accurately determine band intensity. For each sample we determined the ratios of survivin / β-Actin mRNA.

Statistical analysis: All the results were analyzed by SPSS software (version 10). *P* value less than 0.05 was judged statistically significant. Hematological data of cases including WBCs, and Blast percentages are expressed as mean ± standard deviation (SD). Chi and Fisher exact test were performed for testing the significance of results. Student's t test was used for comparison of independent variables.

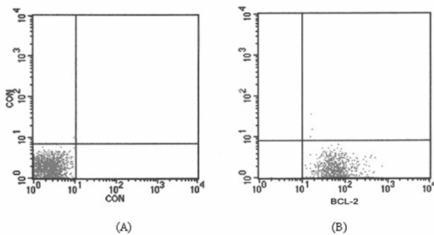
Comparison of mean values of studied variables among different subgroups was done using ANOVA test. Pearson's correlation coefficient was used to quantify the relationship between the variables under study.

RESULTS

Patients' characteristics and clinical data*:* Our study group consisted of 30 patients with AML and 10 healthy subjects as the control group. Forteen of our patients were males and sixteen were

females; age range was between 24 and 58 years (mean 42.87 ±9.8). According to French-American-British (FAB) classify-cation: 8 cases (27%) were M1, while 5 cases were M2 (17%), 3 cases (10%) were M3, 7 cases (23%) were M4, and 7 cases (23%) were M5. White blood cells count, Peripheral blood and bone marrow Blast percentages of AML samples are listed in Table 1.

Bcl-2 expression percentage and mean fluorescence index in AML patients: A broad range of Bcl-2 protein expression was seen in all FAB types. Figure 1 shows in panel (a) the use of a negative isotypic control for adjusting the channel number and also for determining the non-



significant fluorescence. Panel (b) shows an example of Bcl-2 % positive expre-ssion in a studied case of AML.

Figure -1: Flow cytometry analysis of bcl-2 protein expression

a) The negative isotypic control showing the non-significant fluorescence
b) Positive expression of bcl-2 in a case of AML

Bcl-2 protein was detected in all 30 AML cases with a mean of 50% positive cells as shown in table 2 (range: 11–82%). The expression of bcl-2 in AML patients was significantly higher than that in controls (mean=18%, range: 5-57%) ($P < 0.001$). The percentage of bcl-2+ cells was significantly higher in M1 (60%), M2 (54%) and M3 (56%) than in M4 (43%) and M5 (40%) types ($p=0.016$), according to French-American-British classification.

The mean fluorescence index MFI ranged from 4.2 to 35.7 with a mean value of 14.1. The mean bcl-2 MFI was significantly higher ($P = 0.004$) in M1 (20.5) than in M2 (11.9), M3 (11.5), M4 (11.0) and M5 (12.4) cases.

Survivin mRNA expression in AML patients:

Expression of *Survivin* mRNA was detected by RT-PCR in AML patients. The gene products of β Actin and Survivin were 168bp and 206bp, respectively as presented in Fig. 2. Out of 30 patients in subcategories M1 through M5 AML of FAB classification; only 23 patients (76.6 %) showed detectable levels of Survivin expression, while none of the control group showed Survivin expression. Survivin expression in AML cases compared to controls was significantly different ($P=0.000$) as statistically calcu-lated by Chi squared test using data presented in Table 3.

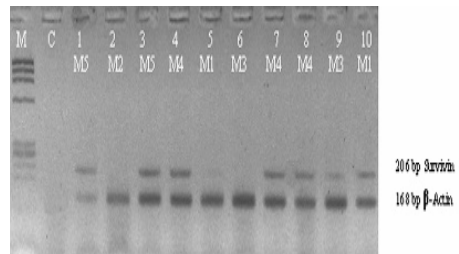


Figure-2: The expression of Survivin mRNA in AML cases. The gene product of Survivin was 206bp, and the gene product of β-Actin was 168bp. **M:** ϕ x174/ *Hae*III DNA marker, C: negative control, 1-10: different AML samples.

Differences in expression between patient subgroups according to the FAB subtype were observed. The expression of

Survivin mRNA varied in correlation with the subgroup as indicated in Table 3. For M1 AML cases seven out of eight patients were positive for Survivin expression, similarly all M4 and M5 cases were positive while only one case of M2 and another of M3 showed bands of Survivin mRNA (Table-3). The differences in expression between subtypes were statistically significant (*P*=0.001) as calculated by chi squared test.

The relative intensity of Survivin expression was analyzed quantitatively in AML samples showing expression bands. The expression level was calculated as a ratio between Survivin mRNA and β-Actin mRNA and the expression levels were plotted in Figure-3. Different expression levels of Survivin mRNA could be observed between different pathologic grades; Three of the 5 subgroups of AML patients (M1, M4, M5) had higher mean levels of Survivin mRNA expression of (mean range: 0.56-0.81) as compared to subgroups M2 and M3 (mean range:0.04-0.1, Table 3).

Levels of Survivin expression varied even within the same diagnostic subcategory. The highest peak of Survivin expression among all AML subcategories was that of M5 as presented in Table 3. An independent *Student t* test was performed to compare the levels of Survivin mRNA in highly expressive subgroups (M1, M4 and M5) with that of the low expressive subgroups (M2, M3), the difference was statistically significant (t= 7.2, p=0.000). In addition one way ANOVA test between each individual AML subgroup showed high significant difference (p=0.000, Table 3).

Aven mRNA expression in AML patients: Expression of Aven mRNA was detected by RT-PCR in AML patients. None of the examined samples showed diagnostic bands for Aven although all samples were positive for the internal control house keeping gene β actin.

Correlation of bcl-2 with Survivin expression and other hematological parameters: The survivin-positive AML cases showed a higher percentage of bcl-2 expression and a significantly higher Bcl-2 MFI in comparison with survivin-negative ones (p= 0.01). Bcl2 expression index (MFI) in relation to other studied parameters is presented in Table 4. There was a high significant correlation between Bcl2 MFI and peripheral blast cells (r= 0.45, p=0.01) as well as with BM blasts (r=0.42, p=0.02) in total AML cases. There was also a significant correlation between Bcl-2 MFI and white blood cells (r=0.87, p=0.009) in M5 subtype.

The survivin expression level was significantly correlated with BCL-2 MFI in total number of cases (r=0.39, p=0.03). Stratification of samples according to FAB classification showed a significant correlation between Bcl2- MFI and survivin expression level in M1 subtype (r=0.8, p=0.017) while it was highly significant in cases of M4 subtype (r=0.97, p=0.00).

Correlation between Survivin expression levels and hematological parameters:

The hematological parameters of the studied cases in relation to Survivin expression levels in AML cases in different subgroups are displayed in Table 5. A significant positive correlation was found between Survivin expression level and peripheral blood (PB) blast % in M5 subgroup only (r = 0.8, p=0.02). However, the correlation between Survivin expression level and bone marrow (BM) blast % exists but does not reach the level of significance (r =0.69, p=0.08). The Survivin expression level

did not correlate with WBCs count in all subgroups of AML cases.

R: pearson's correlation, p: Sig. (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

Recently, there has been great interest in apoptosis, or programmed cell death, the mechanism by which cells essentially suicide (28). Many inhibitors of apoptosis are known to contribute to tumorigenicity and increased spread of tumor cells (29). Bcl-2 plays a key role in the regulation of apoptosis. The *bcl-2* oncogene blocks apoptosis in various cell types and is expressed in both normal and malignant haematopoietic cells including leukemic blasts derived from acute myeloid leukemia (AML) (30, 31).

The expression of bcl-2 protein was evaluated by quantitative flow cytometry in 30 Egyptian cases of acute myelogenous leukemias (AML). Similar to other studies (25, 30-34) all patients were positive for bcl-2expression. Variable percentages of bcl-2+ cells (11-82%) were found and similarly the mean fluorescence index (MFI) of the detected *bcl-2* protein expression in positive cells varied form 4.2-35.7.

Concerning the FAB classification, our data support the results of previous studies indicating significantly lower levels of *bcl-2-* expression in myelo-monocytic AMLs (M4 and M5, Table 2). Thes studies carried out by Perwit et al., (32) and Karakas et al., (33) stated that the bcl-2 levels decrease with granulo-cytic and monocytic differentiation. On the other hand, Campos et al., (34) reported a significantly higher percentage of *bc!-2+* cells in these FAB subtypes. However, our results confirm the general observation of higher *bcl-2* expression in more immature cells (30). These higher bcl-2 levels are postulated to give a survival advantage and confer some degree of drug resistance to the least differentiated blast populations (32). We found a significant correlation between bcl-2 MFI and PB blast % as well as BM blast% in total cases. In addition, bcl-2 MFI correlated with WBCs in M5 subtype.

Survivin is a new member of the inhibitor of apoptosis protein (IAP) family (9). This gene inhibits apoptosis by blocking the effects of caspase-9, which is activated in extrinsic and intrinsic pathways (9, 35, 36). Survivin is expressed in many malignant tumors, including breast, lung, stomach, colon and pancreatic cancers, bladder tumors, malignant lymphoma, and neuroblastoma (37). It is not usually present in normal tissues and is rarely found in mature tissues (35). Thus, Survivin expression is likely to be an important prognostic factor in tumor malignancy.

Aven has recently been identified as an anti-apoptotic protein. Higher levels of Aven mRNA are seen in childhood acute lymphoblastic leukemia than in control patients, suggesting that Aven expression can predict prognosis in child hood ALL (21). The prognostic value of Survivin and Aven in hemopoietic neoplasias has not been as widely studied as in solid tumors. Although the data are limited, the prognostic value of Survivin has been studied in some hemopoietic malignancies such as high-grade lymphomas and leukemias (38-44). While Aven expre-ssion is less frequently studied in hemo-poietic neoplasias.

We therefore used reverse transcription-polymerase chain reaction (RT-PCR) to investigate the expression of

Survivin and Aven mRNA in a group of AML cases. The fidelity of mRNA extraction and RT was tested using oligonucleotide primers for β-actin a “housekeeping” gene. The β-actin primers as described by Raff *et al*., (45) do not amplify genomic DNA and therefore provide absolute evidence that RT has been successful.

In the present study 30 patients of acute myeloid leukemia -subdivided into M1-M5 FAB subtypes- and 10 control subjects were examined by RT-PCR for Survivin and Aven expression. The expression of Survivin mRNA was detected in 23 patients out of 30 (76.6%), while none of the control group showed detectable expression. These results are in a good agreement with Adida *et al.,* (39) who reported Survivin expression in 60% of a series of 125 cases of *de novo* AML.

These results are supported by the study of Carter et al., (40) demonstrating expression of Survivin protein by Western blot analysis in acute myeloid cell lines and in primary AML samples. As opposed to western blot analysis and immunohistochemistry, the PCR based approach used in our study is exquisitely sensitive and may detect gene transcripts even in a single cell or cell cluster with a comparable specificity (46).

Other studies proved that Survivin was expressed in all AML cell lines and most of the leukemia cells but not in normal peripheral blood mononuclear cells and/or bone marrow cells (39, 42, 42, 47, 48). Similarly, Paydas et al., (27) showed Survivin expression to be higher in study group of AML and ALL cases (in 35 patients, 54%) than in controls. In some studies Survivin-expressing leukemias have been found to have a worse prognosis compared with non-expressors (38, 42, 43, 49).

In the current study, the expression of Survivin mRNA among AML subtypes showed two distinct groups; a highly expressing group (M1, M4, M5) as 7 patients out of 8 in M1 cases and all cases in M4 and M5 were positive for Survivin expression. The second group is a lower expressing one (M2 and M3) as 1 patient out of 5 and 1 patients out of 3, respectively, showed positive expression bands. There was a statistically significant difference between expressing cases in different FAB subtypes (p=0.001). This variation within the different subtypes of AML indicates that the expression of Survivin mRNA might reflect the disease progression.

The Survivin expression was previously studied by immunohistochemistry in bone marrow cells from patients with chronic myelomonocytic leukemia (CMML) to evaluate possible abnormalities in comparison with other myelodysplastic (MDS) and myeloproliferative syndromes (18). Invernizzi et al., (18) found that in CMML there was no correlation between Survivin expression and blast cell percentage, FAB or WHO subgroups. In present study, it was analyzed quantitatively the Survivin mRNA and β-actin mRNA within AML samples subtypes. The highly expressing subgroup of AML patients (M1, M4, M5) had higher mean levels of Survivin mRNA expression (mean range: 0.56-0.81) as compared to subgroups M2 and M3 (mean range: 0.04 -0.1). In accordance with our study, Mori et al., (36) found that Survivin expression was lower in patients with M3 acute promyelocytic leukemia than in patients with other types of acute leukemia. Yet, No other studies have reported the frequency of Survivin expression in AML subtypes except the study of Invernizzi et

al., (18) which was concerned with the FAB classification within CMML cases.

Our findings demonstrate that in M5 subgroup patient -according to FAB classification- a significant correlation was between Survivin expression level and PB blast % and also the correlation existed with BM blast %. This may provide useful information for the diagnosis and prognosis of AML subgroups. While in another study they found that Survivin expression correlated with lower WBCs count and favourable /intermediate cytogenetics in B-cell lymphomas (38). Our study revealed the absence of Aven mRNA in both AML cases and controls, this finding is contradictory to the study of Paydas et al., (27) who examined Aven expression by quantitative real-time PCR in AML and ALL patients and found a mean level of expression of 0.54 for Survivin and a mean level of 0.07 for Aven. As previously mentioned, the regulation of apoptotic cell death has a profound effect on the pathogenesis and progression of cancer cells and the correlation between antiapoptotic signals are important in the development and prognosis of hematological malignancies (50, 51). In the present study, the survivin-positive AML cases showed a higher percentage of bcl-2 expression and a significantly higher Bcl-2 MFI in comparison with survivin-negative cases (p= 0.01). Similarly, a Chinese study (52) showed that survivin-positive AML cases had a significantly lower Fas and higher bcl-2 expression in comparison with survivin-negative ($P < 0.01$ and $P < 0.001$, respectively).

Our findings suggest that up-regulation of Bcl-2 and Survivin expression in AML cases may be involved in its pathogenesis. They may provide a useful tool for molecular diagnosis of acute myeloid leukemias. In addition novel therapeutic intervention strategies for treatment of leukemia could use the anti apoptotics; survivin and bcl2 as therapeutic targets as previously suggested by Mesa and Kaufmann (53).

Table –1: Clinico-pathological characteristics of AML samples analyzed

AML cases	WBCs* ($x10^9$/L)	PB blast %*	BM Blast %*
Total(n=30)	51.45±27.52	49.03±27.93	60.50±26.83
M1 (n=8)	66.95±29.77	86.50±6.57	93.13±2.36
M2 (n=5)	42.90±26.59	43.60±10.04	61.40±6.15
M3 (n=3)	44.80±27.36	30.67±1.15	39.00±1.15
M4 (n=7)	30.15±6.73	26.43±4.39	33.29±4.50
M5 (n=7)	64.00±27.30	40.57±29.80	56.86±29.95

* data are presented as means ± standard deviation (SD)

Table –2: Levels of Bcl-2 in studied AML samples and controls

AML cases	Bcl-2 Expression %			ANOVA	MFI			ANOVA
	Mean	SD	SE		Mean	SD	SE	
Total (n=30)	50	18.2	3.3	F=1.60	14.1	7.7	1.4	F= 2.27 P=0.089
M1 (n=8)	60	13.5	4.8	P=0.205	20.5	9.3	3.3	
M2 (n=5)	54	15.7	7.0		11.9	5.4	2.4	

M3 (n=3)	56	19.3	11.2		11.5	6.9	4.0	
M4 (n=7)	43	19.2	7.3		11.0	5.1	1.9	
M5 (n=7)	40	19.9	7.5		12.4	6.8	2.5	

SD: standard deviation, SE: standard error

Table -3: Levels of Survivin gene expression in studied AML samples

AML cases	Survivin Expression		Survivin level Survivin/ β- actin ratio			ANOVA
	Negative	Positive	Mean	SD	SE	
Total (n=30)	7	23	0.49	0.33	0.06	
M1 (n=8)	1	7	0.56	0.28	0.09	
M2 (n=5)	4	1	0.04	0.08	0.04	
M3 (n=3)	2	1	0.1	0.17	0.1	
M4 (n=7)	0	7	0.57	0.10	0.04	F=17.995 P=0.000**
M5 (n=7)	0	7	0.81	0.12	0.05	

SD: standard deviation, SE: standard error ** Statistically highly significant P<0.001.

Table -4: Correlations between levels of Bcl-2 MFI in studied AML samples with survivin level and other hematological parameters.

AML cases	Bcl2- MFI and survivin level		Bcl2- MFI and WBCs		Bcl2- MFI and PB blast %		Bcl2- MFI and BM blast %	
	r	p	r	p	r	p	r	p
Total (n=30)	**0.39***	**0.03**	0.03	0.86	**0.45***	**0.01**	**0.42***	**0.02**
M1 (n=8)	**0.8***	**0.017**	0.27	0.51	0.12	0.78	0.24	0.55
M2 (n=5)			0.16	0.79	0.35	0.56	0.30	0.62
M3 (n=3)			0.92	0.25	0.64	0.55	0.41	0.72
M4 (n=7)	**0.97****	**0.00**	0.21	0.65	0.35	0.44	0.39	0.39
M5 (n=7)	0.26	0.57	**0.87****	**0.009**	0.03	0.94	0.02	0.96

R: pearson's correlation, p: Sig. (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed)

Table -5: Correlations between levels of Survivin expression in studied AML samples and other hematological parameters.

AML cases	Survivin Expression and WBCs		Survivin Expression and PB blast %		Survivin Expression and BM blast %	
	r	p	r	p	r	p
Total (n=30)	0.130	0.494	0.134	0.480	0.119	0.531
M1 (n=8)	0.455	0.258	0.357	0.385	0.236	0.574
M4 (n=7)	0.299	0.515	0.470	0.288	0.488	0.267
M5 (n=7)	0.228	0.622	**0.826***	**0.022**	0.697	0.082

R: pearson's correlation, p: Sig. (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).

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