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Prognostic Relevance of Telomerase and Bcl-2 in Acute Myeloid Leukemia

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ABSTRACT

Background: Prognosis of AML patients is influenced by both clinical and genetic markers. As therapy and supportive care improves, the intrinsic biologic characteristics of the patient's leukemia become the dominant factor in determining prognosis.

Aim of Work: Is to evaluate of the prognostic relevance of Telomerase activity and serum level of Bcl-2 in AML patients.

Patients and Methods: The study included 63 newly diagnosed cases of acute myeloid leukemia below the age of 60 years. Telomerase activity and Bcl-2 levels were assessed in patients as well as in ten healthy age and sex matched controls. Assessment of telomerase activity was done using PCR-ELISA technique and evaluation of Bcl-2 serum level was done using ELISA. Patients were followed up for 3 years. Assessment of prognostic factors in the present study included three main parameters: cytogenetic abnormalities (20 cases), immunophenotyping (63 cases) and hyperleukocytosis (63 cases). Patients were grouped according to the presence of independent prognostic factors into a poor prognosis group and a non-poor prognosis group. Using this classification half the patients (29 patients: 46%) were categorized in the poor prognosis group.

Results: Thirty six percent of patients had hyperleukocytosis (TLC ≥ 100,000/µl), 38% expressed unfavorable immunophenotypic markers (CD34 positivity and or biphenotypic leukemia's markers), while 3 patients had a poor karyotypic profile (11q 23, t (9; 22), del 5q). The complete remission rate was 57% and the overall median time to CR was 31 days. The 2-year and 3-year overall survival rates were 32.5% and 23.5% respectively. While the 2 and 3-year disease-free survival rates were 21.6% and 18% respectively.

Patients in the poor prognosis group showed an inferior 2-year disease-free survival (12% versus 34%; *p*=0.02). The median level of telomerase activity for AML patients was 0.40 U was significantly higher than controls. Higher telomerase values were significantly associated with response rate (*p* 0.01). Lower levels of telomerase activity were associated with a significantly better disease free survival at 1 year when compared to higher levels (34% versus 10%; *p*=0.012). There was also a highly significant association between higher telomerase activity and the poor prognosis group (*p*=0.0001).

The median serum level of Bcl-2 for patients in the present study was 204 U/mL which was significantly higher compared with controls (*p* 0.05). Higher levels showed significant correlation with bad prognostic group (*p*=0.0001), bad immunophentypic profile (*p*=0.011) and hyper-leucocytosis (*p*=0.000l). Higher levels were associated with lower response rates (*p*=0.06). Lower levels were significantly associated with better DFS at 1 year (*p*=0.005) as well as better 2 year survival, although in the latter the difference did not reach significance (*p* 0.07). Patients with more than one poor prognostic criterion had a tendency for lower overall survival rate at 1 year (24% versus 42%; *p*=0.094) and significantly lower disease-free survival rate at 1 year (24% versus 50%; *p*=0.036). There was a significant correlation between telomerase activity levels and Bcl-2 level in the serum of AML patients (r=0.623, p<0.0001).

Conclusion: Telomerase activity and Bcl-2 levels correlate significantly with disease-free survival in AML patients. Prospective studies with more numbers of patients will be required to confirm their prognostic values especially in specific subsets of patients as those with normal cytogenetics where there is still debate about the role of intensive therapy with stem cell transplant.

Key Words: *AML – Telomerase – Bcl-2.*

INTRODUCTION

The development of acute myeloid leukemia (AML) is associated with accumulation of acquired genetic alterations and epigenetic changes

in hematopoietic progenitor cells that alter normal mechanisms of cell growth, proliferation, and differentiation with consequent uncontrolled proliferation of clonal neoplastic hematopoietic precursor cells and impaired production of normal hematopoiesis **[1]**. Approximately 50% to 75% of adults with AML achieve complete remission (CR) with the deoxycytidine analog cytarabine and an anthracycline antibiotic, such as daunorubicin or idarubicin, or the anthracenedione mitoxantrone. However, overall, only 20% to 60% of patients enjoy long-term disease-free survival (DFS) depending on risk factors at diagnosis. The majority of patients die of their disease, primarily because of persistent or relapsed AML **[2]**.

Post-remission therapy is a sine qua non for curing AML as the median DFS for patients who receive no additional therapy is only 4-8 months, however, the optimal dose, schedule, and number of cycles of consolidation chemotherapy for most patients with AML who achieve CR have not been established. In younger patients, cycles of intensive consolidation chemotherapy, often with, but not limited to, high-doses of cytarabine, prolong DFS and OS. Stem cell transplantation is anther option in high risk patients **[3-6]**.

Various factors affect the prognosis of AML including age, Immunophenotype, WBCs count at diagnosis, response to induction therapy and chromosomal abnormalities which are considered the most important prognostic factors currently used for choice of post-remission treatment **[6-11]**.

Chemotherapy kills cancer cells primarily by inducing apoptosis. Therefore, modulation of the key elements of apoptosis signaling directly influences therapy-induced apoptosis. Telomerase is a specialized ribonucleoprotein complex that is responsible for the synthesis and maintenance of telomere repeats. The latter are DNA-protein complexes at the ends of linear eukaryotic chromosomes which maintain chromosomal stability and integrity and protect chromosomal ends against fusion, degradation by exonucleases and recombination events. Unlike embryonic cells, telomerase expression is low or absent in most human somatic tissues and in adult cells. The catalytic protein subunit of TERT is the key determinant of the enzymatic activity of human telomerase **[12]**. The clinical relevance of telomeres is that a cancer cell, unlike a normal cell, can repair eroded telomeres. Telomerase activity is expressed in varying degrees in most primary tumors. The overall importance of telomerase in the pathogenesis of AML has recently been confirmed by the demonstration that hTERT is necessary for growth of primary AML cells in a mouse model **[13]**. However, considerable work has been undertaken to determine whether telomerase activity can further refine these prognostic data.

The pathways responsible for adult tissue homeostasis are governed significantly but not exclusively by Bcl-2-family proteins (pro and anti apoptotic) **[14]**. The Bcl-2 regulation pathway is also called the intrinsic or mitochondrial pathway of caspase dependent apoptosis. As such Bcl-2 genes are regarded as potential oncogenes in view of their function in apoptotic cell death and the consequence of perturbation of the delicate homeostasis of cell population growth. In this case the effect is not achieved by increasing the rate of cell proliferation but by reducing the rate of cell death **[15,16]**. Bcl-2 overexpression in haematopoietic lineages yields excess B, T and myeloid cells that are refractory to diverse cytotoxic insults **[17-19]**.

The aim of this study is to estimate levels of telomerase and Bcl-2 in patients with de novo AML and to correlate it with clinical and biological factors which are known to influence prognosis.

PATIENTS AND METHODS

This study comprised a total of 63 newly diagnosed cases of acute myeloid leukemia who presented to the Medical Oncology department at the National Cancer Institute, Cairo University between February 2002 and April 2004. We followed up the patients for 3 years. Pretreatment assessment included:

Full history and clinical examination:

Hematological studies: Complete blood count with differential, Bone marrow examination with cytochemical stains needed for proper diagnosis (eg; Sudan black, myeloperoxidase, non specific estrases).

Routine biochemical investigations that included hepatic and renal profiles (bilirubin, ALT, AST, alkaline phosphatase, urea and cre-

atinine in addition to tumor lysis syndrome panel including uric acid, Ca, K, Mg and Po4.

Immunophenotyping: Was done using Flow-cytometry Partec III from DAKO. A wide panel of monoclonal antibodies was used as part of the diagnostic procedure and for the documentation of surface or cytoplasmic marker expression by leukemic cells. CD34, CD33, CD11, CD13, CD14, CD15, MPO, HLA-DR, CD10, CD19, CD22, CD7, CD41, CD16, CD56. All monoclonal antibodies were obtained from DAKO (Denmark).

Cytogenetic studies: Conventional cytogenetic analyses were carried out on unstimulated bone marrow or peripheral blood cultures. Metaphases were trypsin/Giemsa-banded. Karyotypic abnormalities were described according to the specifications of the International System for Human Cytogenetic Nomenclature. For cytogenetic analysis an automated karyotyping system (Quips, Vysis, USA) was used.

Imaging studies: Chest X-ray, abdominal ultrasonography and CT scan when appropriate in addition to ECG and Echocardiography.

Telomerase activity was assessed by Telomerase PCR ELISA utilizing the telomeric repeat amplification protocol (TRAP), developed by Boehringer Mannheim (Germany) which is an extension of the original method described by Kim et al. **[20]**. TRAP assay is a 2-step process in which the telomerase-mediated elongation products are subsequently amplified by PCR to allow highly sensitive detection of telomerase activity.

Serum Bcl-2 level was assayed using ELISA method (Oncogene Research Products, Cambridge, MA).

Treatment plan:

Remission induction:

Patients received the conventional 7 and 3 regimen, consisting of cytarabine 100 mg/m^2 continuous infusion over 24 hours for 7 days and doxorubicin 45 mg/m^2 IV shot for 3 days. If 14 days after induction therapy, the result of bone marrow aspirate revealed partial remission or no response to therapy, a second induction course was given consisting of the same regimen used in the induction course.

Consolidation/ early intensification therapy:

Patients who achieved complete remission received consolidation therapy consisting of:

- Two cycles of cytarabine 100 mg/m^2 continuous infusion over 24 hours for 5 days and Doxorubicin 45 mg/m^2 IV shots for 2 days.
- Followed by two cycles of cytarabine 1 gm/m^2 infusion over 2 hours for the first 3 days and Mitoxontrone 12 mg/m^2 intravenous infusion over 2 hours on days 3, 4, 5.
- Patients who achieved complete remission and were eligible for bone marrow transplantation were referred for Allogenec HCT.

Maintenance therapy:

All patients were kept under follow-up without maintenance therapy.

Statistical analysis:

Descriptive statistics was presented in frequency tables, means and standard deviations whenever appropriate.

Analytical tests used included:

- Chi-square test for comparing two quantitative variables.
- Survival analysis and analysis of duration of complete remission were done using Kaplan Meier analysis.
- Correlation between quantitative variables is done by the R-test diagrammatically represented by scatter dot diagram.

Significance level of less than 0.05 was used in all statistical tests.

RESULTS

This study involved 63 patients with age range of 16-63 Y and a mean of 33.9±11.7 year. Ten age and sex matched healthy individuals were included as a control group. Patient characteristics are illustrated in Table (1).

Overall 24 cases expressed immunophenotypic markers of poor prognosis. In all cases at least 2 myeloid markers were expressed. Twenty one cases were found to be CD34 positive while 5 cases of biphenotypic leukemia. All biphenotypic cases co-expressed B lineage markers (CD22 in all, cytoplasmic Ig in one case). Two

cases were both CD34 positive and expressed CD22. Immunophenotypic characteristics of AML patients studied are shown in Table (2). FAB classification and cytogenetics are shown in Tables (3,4).

Toxicity:

Scoring of treatment toxicity was done according to WHO criteria. Overall the most common non hematological toxicity was stomatitis, which was observed in almost 60% of patients. Stomatitis was also the most common form of GIII-IV toxicity (23.5%). Mild to moderate nausea and vomiting and alopecia were present in 47% patients. Twenty five percent of patients experienced mild diarrhea, whereas III-IV was recorded in 2 patients Furthermore, 21 patients (33%) presented with liver toxicity with 6 of them who experienced severe liver toxicity grade III/IV. Moreover, Six patients (10%) experienced sudden cardiac collapse (3 cases of acute heart failure and 3 cases of sudden death due cardiac arrhythmias), Table (5).

Neutropenia and supportive therapy:

The maximum nadir was reached after a mean of 14.86±4.40 days. The mean duration of neutropenia (neutrophils < 1000/cmm) was 13.56±8.68 days.

Fifty nine patients (93%) received antibiotics during induction chemotherapy, while 46 patients (73%) required antifungal therapy. The mean duration of antibiotics was 22.92±10.14 days while the mean duration of antifungal therapy was 14.43±9.13 days. The duration of neutropenia correlated significantly with the duration of antibiotics (p=0.003) but did not achieve statistical significance when correlated with the duration of antifungal therapy (p= 0.32).

Causes of death:

The main cause of death was related to uncontrolled infection (36% of patients). This was followed by cerebral hemorrhage (13%), while 6 patients (10%) died of disease progression with one patient dying from CNS infiltration (Table 6). Ten percent of cases died from cardiac and circulatory collapse, while 3 patients died from documented heart failure, another 3 due to arrhythmia (a result of electrolyte imbalance from toxicity) and one from hemorrhagic shock. Two patients died from liver failure (hepatic coma).

There were 16 cases of early death (death prior to evaluation of response). Six cases died from febrile neutropenia, 5 cases died of cerebral hemorrhage and 1 patient suffered toxic death as a result of GIII diarrhea leading to circulatory failure.

Response rates:

Complete remission (CR) was achieved in 32 patients after receiving the 1st cycle of induction. Two patients died immediately after achieving CR. Four patients required a second cycle of induction to achieve CR bringing the overall CR rate to 36 patients (57%). Eleven patients achieved partial response after their first cycle while 4 patients showed disease progression. Relapse rate was 30% (11 cases out of 36).

Time to first CR:

Overall median time to first CR was 31 days (14 to 75). There was no difference in the time to first CR between the group of the patients classified as poor prognosis and the non-poor group, (34 Vs. 40 days; p=0.437). No correlation was found between time to first CR and the individual prognostic factors, hyperleukocytosis or poor prognosis immunophenotypic markers (p=0.606 and p=0.254, respectively).

Disease free survival and overall survival:

The 2 and 3-year disease-free survival were 21.6% and 18% respectively for the entire group of patients enrolled in the study.

The 2 and 3-year overall survival rates were 32.5% and 23.25% respectively for the entire group of patients enrolled in the study.

When we compared the outcome of the poor prognosis group to non-poor prognosis group we found no statistical differences in overall survival at 2 years (29% versus 34%; p<0.094). As regards disease-free survival, however, patients classified in the poor prognosis group showed an inferior 2-year disease-free survival (12% versus 34%). This difference was found to be statistically significant (p=0.02).

Telomerase activity:

Telomerase level:

The mean telomerase activity level in leukemic cells of the present study was found to be 0.41 U±0.04 with a median of 0.40 U (0.38 to 0.56). There was a statistically significant difference between the median telomerase ac-

tivity level in the control group and the AML group ($p<0.05$), Table (6).

Correlation between telomerase activity and different prognostic factors:

Telomerase level was inversely correlated with age of patients but statistical significance was not achieved ($p=0.157$). In addition, direct correlation with total leukocytic count was shown but the correlation failed to achieve statistical significance ($p=0.238$). Moreover, there was no statistically significant correlation between telomerase levels and hyperleukocytosis (> 100,000/cmm) or immunephenotypic characteristics of poor prognosis, (CD34 positivity, biphenotypic leukemia), ($p=0.238$, 0.275) respectively. We were unable to correlate telomerase with cytogenetic abnormalities due to an inadequate number of patients; however, all patients with poor cytogenetic profile (3) had elevated telomerase activity levels.

There was a statistically significant correlation between telomerase and the prognostic grouping of the patient. Twenty five patients out of 26 in the poor prognosis category expressed higher telomerase level while only one patients out of 34 in non-poor prognosis group expressed higher telomerase level of activity ($p=0.0001$).

Correlation between telomerase response, DFS and OS:

Although there was a significant correlation between higher telomerase values and achieving CR ($p=0.019$), there were no correlation between the telomerase level and time needed for CR ($r=0.200$) Fig. (1). Lower levels of telomerase were associated with better DFS at 1 year when compared to higher levels (34% Vs. 10%; $p=0.012$) Fig. (2). The 2-year overall survival was higher in patients with levels of telomerase below 0.4 (42%) than those patients with telomerase level higher than 0.4 (34%). The difference was not statistically significant ($p=0.134$) (Fig. 3 and Table 7).

Bcl-2:

Level:

The mean Bcl-2 level in the serum was 232.8±124.4 SD while the median was 204 (50 to 44.0). There was a statistically significant difference between Bcl-2 serum levels in the control group (70±21 U/mL) and patients ($p<0.05$).

Correlation between Bcl-2 level and prognostic factors:

Although a strong inverse association between Bcl-2 level and the age could be detected, it did not reach statistical significance ($p=0.078$). No statistically significant association was found between telomerase activity and TLC ($p=0.238$. Bcl-2 levels were correlated with hyperleukocytosis and the results were highly significant ($p<0.0001$). It also correlated significantly with poor prognosis immunophenotypic profile ($p=0.011$). We were unable to correlate Bcl-2 with cytogenetic abnormalities due to an inadequate number of patients; however, all patients with poor prognosis karyotype (3) had elevated Bcl-2 levels.

There was a statistically significant correlation between Bcl-2 level and the prognostic grouping of the patient. All patients in the poor prognosis group expressed higher levels of Bcl-2, ($p=0.0001$) while only 3 patients from the non-poor prognosis group had higher Bcl-2 levels.

Correlation between Bcl-2 and response, DFS and OS:

The inverse association between higher levels Bcl-2 and CR rate almost achieved statistical significance ($p=0.06$). There was no significant association between Bcl-2 level and time needed to achieve complete remission. Lower levels of Bcl-2 were associated with significantly higher DFS at 1 year when compared to patients with higher levels (37% Vs. 11%; $p=0.005$). The 2-year overall survival was 44% in patients with Bcl-2 levels < 200 U/mL and 24% in those presented with higher Bcl-2 levels. The difference, although almost double, did not achieve statistical significance ($p=0.078$) (Table 8).

Correlation between telomerase and Bcl-2:

There was a significant correlation between telomerase level and Bcl-2 ($r=0.623$ highly significant; $p<0.0001$). We also examined whether the cumulative assessment of telomerase activity in conjunction with Bcl-2 levels and the presence of adverse prognostic factors would correlate better with disease outcome. We found that patients with more than one poor prognostic criterion had a tendency for lower overall survival rate at 1 year (24% versus 42%; $p=0.094$). The disease-free survival was significantly associated with the number of poor

prognostic criteria with a 1-year disease-free survival of 23% in patients with more than one poor prognostic criterion and 50% in those who showed only one criterion (p=0.036).

Table (1): Patient's characteristics.

Character	No (%)
Age (Y):	
Range	16-63
Mean	33.9±11.7
Sex:	
Male	27 (42.9%)
Female	36 (57.1%)
Ratio	1:1.3
WBCs x10^9/l:	
< 25 x10^9/l	23 (36.5%)
25-100 x10^9/l	17 (27.0%)
> 100 x10^9/l	23 (36.5%)
HB gm/dl:	
< 8	44 (69.8%)
≥8	19 (30.2%)
Platelets x10^9/l:	
< 25	18 (28.6%)
≥25	45 (71.4%)
Bone Marrow Blasts %:	
< 75%	42 (67%)
> 75%	21 (33%)
Symptoms:	
Fatigue	48 (76.2%)
Fever	42 (66.7%)
Bony aches	38 (61.3%)
Bleeding	13 (21.0%)
Signs:	
Splenomegaly	27 (43.6%)
FUO*	25 (39.7%)
Hepatomegaly	18 (28.6%)
Mucositis	11 (17.7%)
Lymphadenopathy	8 (12.9%)

*FUO= Fever of unknown origin.

Table (2): Immunophenotypic character of AML patients.

Cluster of Designation	No (%) of positive cases
CD34	21 (33%)
MPO	34 (58.6%)
CD33	42 (66.7%)
CD13	45 (72.6%)
CD14	10 (15.9%)
CD11	1 (1.6%)
CD15	3 (4.8%)
CD45	13 (20.9%)
CD7	8 (12.6%)
CD10	4 (6.4%)
CD19	6 (9.5%)
CD22	5 (7.9%)
CD41	1 (1.7%)
CD16	2 (32.2%)
CD56	3 (4.7%)
HLA-DR	34 (53.9%)
Biphenotypic	5 (7%)
Total patients with poor IPT profile	24 (38%)

Table (3): French-american-british (FAB) classification.

FAB	Number of patients (%)
M1	23 (36.5%)
M2	22 (35%)
M4	11 (17.4%)
M5	7 (11%)

Table (4): Chromosomal analysis.

Cytogenetic	Number of patients
Good prognosis:	
t (8; 21)	2
inv (16)	none
Poor prognosis:	
11q 23	none
t (9; 22)	2
del 5q	1
Intermediate prognosis:	
Normal karyotype	10
Other abnormalities	5

Table (5): Non-hematological treatment related toxicity.

Toxicity	Grade I-II Number of patients (%)	Grade III-IV Number of patients (%)
Alopecia	30 (47%)	0 (0%)
Stomatitis	23 (36%)	15 (23.5%)
Nausea/vomiting	30 (47%)	0 (0%)
Diarrhea	16 (25%)	2 (3%)
Hepatic	15 (23.8%)	6 (9.6%)
Cardiac	0 (0%)	6 (9.6%)

Table (6): Median levels of telomerase activity.

Telomerase Mean: 0.41± 0.04 Median: 0.4 (0.38-0.56)	No of patients (%) 63 (100)
< 0.40	37 (58.7%)
≥ 0.40	26 (41.3%)

Table (7): Correlation between telomerase and prognostic category.

Telomerase activity/ number of patients	Non-poor	Poor	p	2-year OS	p	1-year DFS	p
< 0.4 (37)	33	4		42%		34%	
			0.0001		0.134		0.012
≥ 0.4 (26)	1	25		23%		10%	

Table (8): Correlation between serum BCl-2 level and prognostic groups at 2-year OS and 1 year DFS.

Bcl-2 (U/mL)	Poor prognosis group	Non poor prognosis group	*p*	2 year OS	*p*	1 year DFS	*p*
<200	0	31 (100)		44%		37%	
≥200	29 (90.6)	3 (9.4)	0.0001	24%	0.078	11%	0.005

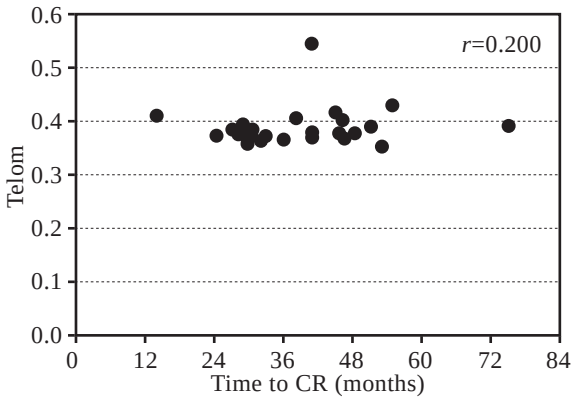

Fig. (1): Telomerase activity and response rate.

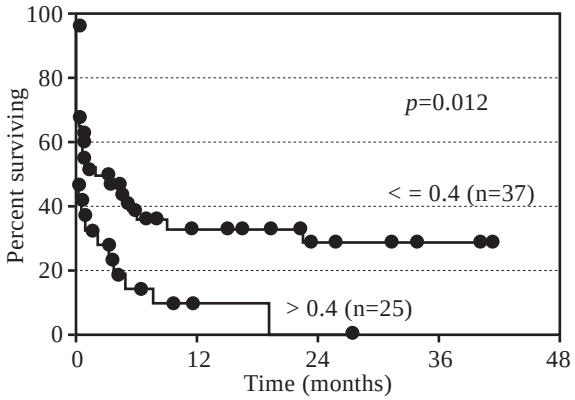

Fig. (2): Correlation between telomerase and disease-free survival.

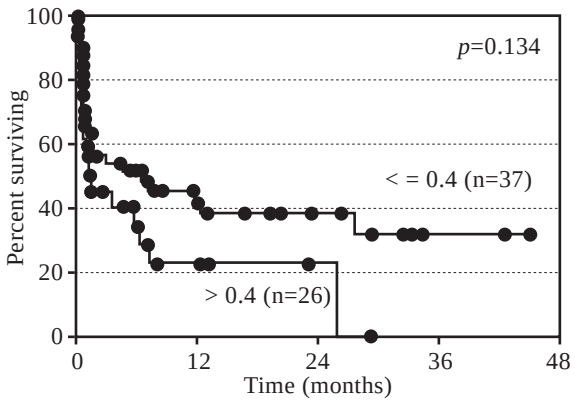

Fig. (3): Correlation between telomerase and overall survival.

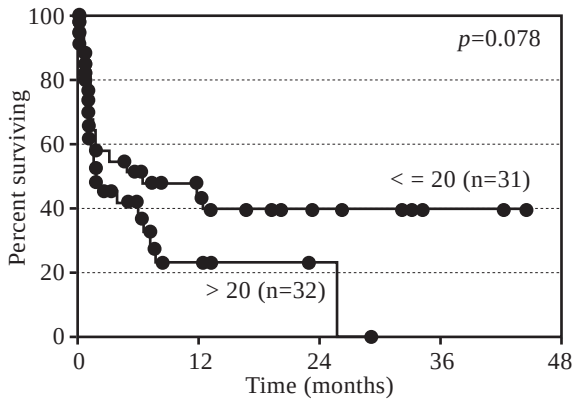

Fig. (4): Correlation between Bcl-2 and overall survival.

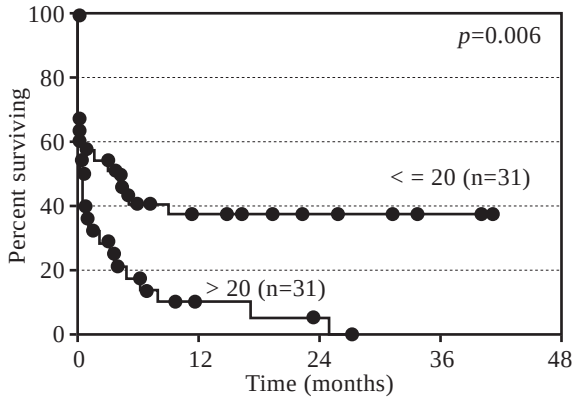

Fig. (5): Correlation between Bcl-2 and disease-free survival.

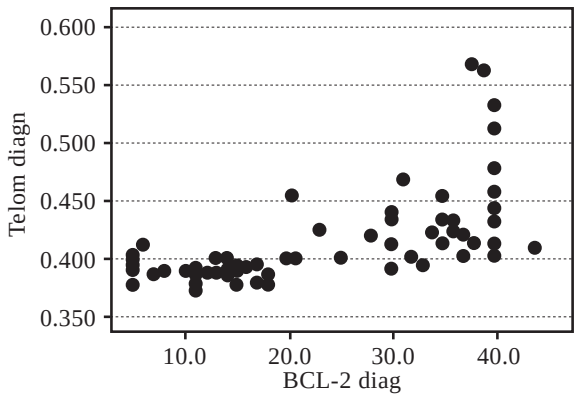

Fig. (6): Correlation between telomerase activity levels and Bcl-2

DISCUSSION

Over the past decade, the application of novel cytogenetic and molecular techniques has markedly improved our knowledge of the pathophysiology of acute myeloid leukemia, resulting in new potential therapies as well as better use of existing ones **[21]**. As therapy and supportive care improves, the intrinsic biologic characteristics of the patient's leukemia became the dominant factor in determining prognosis. Prog-

nosis of AML is mainly affected by cytogenetic risk groupings in addition to total leucocytic count at diagnosis and age **[6]**.

In this study, 63 patients with newly diagnosed acute myeloid leukemia were induced with the standard anthracycline and continuous infusion cytosine arabinoside. Complete remission was achieved in 36 patients (56%) with a 2 Y DFS of 18% and 3-y overall survival of 23%.

Telomerase activity was measured using TRAP assay on peripheral blood samples and it was significantly higher in AML patients compared with normal controls (p<0.05). Patients in the poor prognostic group expressed significant higher levels of telomerase compared with good prognosis group. There was a significant correlation between higher telomerase values and lower CR rates (p=0.019). Lower levels of telomerase were associated with better DFS at 1 year when compared to higher levels (34% Vs. 10%; p=0.012). The 2-year overall survival was higher in patients with levels of telomerase below 0.4 (42%) than those patients with telomerase level higher than 0.4 (34%). The difference was not statistically significant (p=0.134).

Increased telomerase expression in AML was reported by other investigators **[22-28]**. Concurring with our study also is the one conducted by Seol etal., 1998 **[22]** and Huh et al., 2005 **[27]**, who showed no correlation with CD34+, blast counts, white blood cell counts. In the later study, low remission rates were associated with higher telomerase activity with no correlation with time to remission. However, this had not been uniform in all studies as Xu et al., 1998 **[26]**, Verestovesk et al., 2003 **[28]**, did not demonstrate association with response rate or disease free survival. Conversely, Seol et al., 1998 **[22]**, reported that higher response rate were associated with higher telomerase activities. This heterogeneity of results might be explained by variation in the methods used to assess telomerase. Verstovsek et al., 2003 **[28]**, used a modified chain reaction-based, TRAP assay and measured telomerase activity in bone marrow samples. Another study assessed telomerase activity using the TRAP assay with an automatic DNA sequencer to detect and quantitate telomerase activity in peripheral blood samples **[29]**. There were also differences in the samples used with some studies using peripheral blood Xu et al., 1998 **[26]**, others using bone marrow or reporting on cell lines Seol et al., 1998 **[22]** some authors included secondary or relapse leukemia which might have a different biology. Furthermore, differences in the chemotherapy used as well as differences in the median age of patients involved with consequent differences in treatment might explain the inconsistent prognostic results.

Our study also demonstrated higher serum level of Bcl-2 compared with the control group (p<0.05). Significant higher levels were demonstrated in those with hyperleukocytosis (p< 0.0001) and poor prognosis immunophenotypic profile (p=0.011). Several early studies have examined the relation between Bcl-2 expression and individual prognostic factors in AML **[30-32]**. However, an unexpected observation was that among patients with unfavorable prognosis, the prognosis improved as the Bcl-2 level increased **[33]**. This might be explained that the ration of Bcl-2 to other family members which are pro-apoptotic might be more important than Bcl-2only. Overexpression of Bcl-2 was found to correlate with CD34 positivity in many studies of AML patients **[31,34,35]**.

Higher levels of Bcl-2 showed inverse association with CR rate; the results were almost statistically significant (p=0.06). This is in concordance with a number of studies that demonstrated that higher levels of Bcl-2 were associated with low CR rate in a number of studies **[31,32,34-37]**. Lower levels of Bcl-2 were associated with significantly higher 1 Y DFS (p 0.005). There was a trend towards a better survival for lower levels but it did not reach statistical significance (p=0.078). Campos et al., 1993 **[30]**, reported a significantly shorter overall survival (32% v 15% at 2 years; p< 0.005) in patients with high expression of Bcl-2. In this study the percentage of Bcl-2 positive cells, age and the percentage of CD34+ cells were independently associated with poor survival. In another study the three-year overall survival was 10% for patients with high expression levels of Bcl-2 and similar significant differences were observed for the disease free survival **[32]**. The results of these prognostic associations should be interpreted with caution as Bcl-2 has a more complex relationship regarding outcome and prognosis. Some studies

have reported that higher Bcl-2 levels correlate with better prognosis while other studies have reported the reverse **[33,36]**.

A possible explanation for this controversy is that most studies consider Bcl-2 in isolation. However the function of Bcl-2 may depend upon other members of the Bcl-2 family which has the upper hand on the phosphorylation status of Bcl-2. Hence despite the high levels of Bcl-2, the net effect may be pro-apoptotic due to overexpression of the other pro-apoptotic members **[38]**.

In a recent study a high expression of both anti- and pro-apoptotic genes in AML blasts at diagnosis have adverse prognostic impact **[39]**. The authors explained that their finding fits in with the recently defined concept of oncogenic addiction and the primed to death status of some tumor cells **[40]**. This can also explain the higher apoptosis related gene expression variance in AML.

There was a significant correlation between telomerase level and Bcl-2 (r=0.623; p<0.0001). Several studies explored the relation between telomerase and Bcl-2 **[41-44]**. Evaluating a possible association between telomerase activity and Bcl-2 level, we found a significant correlation between telomerase activity levels and Bcl-2 levels (r=0.623 & p<0.0001).

In one of the earliest studies exploring the relationship between Bcl-2 and telomerase activity, Abdel Salam et al. **[45]**, reported that the mean level of Bcl-2 was higher in telomerase positive breast cancer cases than in telomerase negative ones suggesting a possible association between the two markers. A later study confirmed that telomerase and Bcl-2 were independent prognostic factors in Egyptian breast cancer patients and failed to detect a significant association between Bcl-2 and Telomerase **[41]**. As our understanding of the role played by telomerase in tumor progression improves as well as the interplay between various members in the Bcl-2 family of genes, an association between telomerase activity and the anti-apoptotic pathway may emerge.

The important prognostic relevance arise from the fact that they may add to the molecular armentarium including nucleophosmin, FLT-3 and others currently tested to allow for choosing which patient with normal cytogentics will require bone marrow transplantation Beside prognosis two important roles could be potentially useful for these markers either separately or combined which are their use for follow-up of cases to detect early relapse and their potential use as therapeutic targets. Anti Bcl-2 anti sense oligonucleotide is currently under trials. Larger prospective studies with standardization of the techniques and treatment protocols are currently required before definite conclusions could be withdrawn.

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