TELOMERASE ACTIVITY IN ACUTE LEUKEMIA

By

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ABSTRACT

In the present study, we used telomeric repeat amplification protocol assay (TRAP) and an internal telomerase assay standard to detect and quantitate telomerase activity in blood samples obtained from normal (control) subjects and acute leukemia patients.

Telomerase activity was analyzed in 25 acute myeloid leukemia (AML) patients, and 25 acute lymphoblastic leukemia (ALL) patients and ranged from 1.7-171.3 RTA (mean 42.64±57.65) and 2.1-149 RTA (mean 40.73±39.55) respectively relative to the internal standard.

Compared to the age-matched normal levels of telomerase activity in the peripheral blood cells, we determined that 21/25 (84%) of AML patients and 23/25 (92%) of ALL patients had heterogeneously elevated telomerase activity. Acute leukemia patients with high/moderate telomerase activity (AML: n= 12; ALL: n= 16) showed high leucocytic counts and more frequent extramedullary involvement during the disease. In AML, the level of telomerase activity was also associated with French-American-British (FAB) subtypes; patients with AML-M3 had normal to low telomerase activity.

INTRODUCTION

Human telomerase is a cellular RNA-dependent DNA polymerase with an RNA component that provides a template for the synthesis of the tandem arrays of telomeric (TTAGGG) repeats at eukaryotic chromosomes ends to maintain telomeric lengths(1). Telomerase is the only known ribonucleoprotein in human cells with reverse transcriptase activity(2). Without telomeric caps, human chromosomes would undergo end-to-end fusions with formation of dicentric and multicentric chromosomes. These abnormal chromosomes would break during mitosis, resulting in severe damage to the genome and activation of DNA damage checkpoints, leading to cell senescence or initiation of the apoptosis cell death pathway(3).

Telomeric DNA is lost during each cell division due to inability of the lagging strand of DNA to fully replicate the end of linear chromosomes, reffered to as the end replication problem(4). Telomerase, which synthesizes telomeric DNA onto the chromosomal ends, is able to compansate for the loss of terminal telomeric DNA, indeed, it has been proposed that telomere length specifies the number of cell divisions a cell can undergo before senescence(5).

In general, telomerase activity is expressed in human germ cells, and certain stem cells, but essentially is absent in the great majority of normal somatic tissues(6). In normal human somatic cells, expressing low or undetectable telomerase, a progressive shortening of the telomeres is observed every time somatic cells divide contributing to replicative cell senescence. This telomeric shortening, in the absence of telomerase, act as mitotic clock in determining the remaining replicative capacity of the cell(7). In contrast to somatic cells, germ cells maintain the length of telomere through an indefinite number of cell divisions by the expression of telomerase(8).

Acute leukemias are malignant diseases resulting from clonal

proliferation of myloid or lymphoid precursors with arrested maturation(9). In this disorder, the leukemic clone expands in the bone marrow, interferes with normal hematopoiesis and eventually, non-hematopoietic tissues are infiltrated. However, to reach immortality, leukemic cells must also escape the control of cell senescence. Telomerase and telomeres have been shown to be involved in the unlimited proliferation capacity of malignant cells, including leikemias(5,10,11).

Upregulation of the enzyme telomerase is found in over 85% of human cancers(2) and overexpression is commonly found in leukemic cell lines and in BM samples in patients with leukemia(12-14). Genetic experiments have demonstrated that inhibition of telomerase in leukemia cells can result in telomere shortening followed by proliferation arrest and cell death by apoptosis(15-16). This makes terlomerase a target not only for diagnosis but also for development of novel therapeutic agents.

The aim of the present study is to investigate the level of telomerase activity in AML and ALL patients and its implications on the clinical and laboratory data.

PATIENTS AND METHODS

In this study we examined samples from 50 patients with de novo acute leukemia, 25 with AML and 25 with ALL presented to Kasr El-Einy Center Of Oncology And Nuclear Medicine (NEMROCK) during the period from January 2003 to March 2004. Fifteen healthy age and sex-matched persons were enrolled in this study as a control of telomerase activity.

The diagnosis was based on morphological examination of peripheral blood and bone marrow films, cytochemistry and immunophenotyping. Patients were also classified according to the French-American-British (FAB) classification.

Specific laboratory investigation:

Telomerase activity was determined quantitatively by using telomerase PCR-ELISA (telomerase PCR-ELISA kit-Boehringer Mannheim) based on the original TRAP assay(11).

All samples obtained from peripheral blood cells under complete aseptic conditions on heparin and the mononuclear cells were separated using Ficoll-Hypaque gradient. $3x10^6$ cells were counted and cell pellets were stored at -80°C immediately.

Telomerase PCR-ELISA:

Protein extraction: Cell pellets were allowed to thaw on ice, then lysed with ice-cold lysis buffer and incubated on ice for 30 minutes, then centrifuged at 12000g for 30 minutes at 4°C. The supernatant was then collected.

In brief, telomeric repeats were added to a biotin-labelled primer for 30 minutes at 25°C during the first reaction. The mixture was then subjected to 30 PCR cycles at 94°C for 40 seconds, 50°C for 40 sec, and 72°C for 90 seconds (72°C, 10 minutes for the final step). An aliquot of the PCR product was denatured, hybridized to a digoxigenin (DIG)-labelled telomeric repeat specific probe and bound to a streptavidin-coated 96-well plate. Finally, the immobilized PCR product was detected with an anti-digoxigenin-peroxidase antibody and visualized with a colour reaction by using tetramethyl benzidine substrate. The absorbance of the samples was measured at wavelength of 450nm (reference wavelength 620nm) Microplate Reader (Stat-Fax microplate reader) within 30 minutes of addition of the stop reagent.

Statistical analysis:

Data was analyzed using statistical package for science "SPSS version 9" software. Data were presented as number and percentage for qualitative variables but were presented as mean and standard deviation for quantitative continuous variables. The significance of difference between mean values of paired observation was performed using paired t-test.

Chi square (X2) is used for comparison between distributions of patients according to different items.

Correlation co-efficient (r test) is used to rank different parameters against each other.

RESULTS

In the present study, the telomerase levels were estimated in the peripheral blood mononuclear cells (PBMCs) of 25 de novo AML, 25 de novo ALL cases. Their characteristics and hematological data are presented in **table (1)**. Another 15 healthy, age matched subjects were enrolled in this study as a control group.

To estimate the relative telomerase activity levels and to rule out potential false negatives, we used an internal control DNA template (ITAS), which is amplified by the same two PCR primers used in the telomerase activity assay protocol. Then the telomerase activity in each sample was divided by that of the amplified internal standard (ITAS). The mean value of the relative telomerase activity for the control group was 1.01± 0.606 and ranged from 0.2-2.2. Accordingly the cut-off value was calculated as:

Cut-Off value = Mean + 2 SD = 2.2 RTA

We arbitrarily classified acute leukemia patients with elevated telomerase activity as follows: **Low** (above normal and up to 10 relative value), **Moderate** (10 to 50 relative value), and **High** (≥50 relative value).

Table (1): Patients' characteristics and hematological data.

	AML (n=25)	ALL (n=25)
Sex		
M/F	13/12	18/7
Age (yrs)		
Range	15-55	14-40
Mean	35.16±15.82	26.72±9.16
Hb (g/dl)		
Range	4.1-10.0	3.5-11.5
Mean	6.92±1.59	6.09±2.125
TLC ($X10^3/\mu l$)		
Range	2.1-268.5	6-318.5
Mean	53.39±66.3	62.31±82.37
Platelets ($X10^3/\mu l$)		
Range	16.0-58.4	10-110.7
Mean	19.36±22.31	67.68±24.48
Blasts in PB (%)		
Range	63.0-96.0	43-99
Mean	70.48±25.31	57.96±29.62
Blast in BM (%)		
Range	45-96	85-99
Mean	62.34±21.12	92.33±6.52
FAB	17 (M1/M2) 3 (M3) 5 (M4/M5)	13 (L1) 12 (L2)
Immunophenotype		1 (Pro-B) 22 (Pre-B) 2 (T-ALL)

Telomerase activity in acute leukemia patients:

Compared to the normal level of telomerase activity of control group, we determined that 21/25 AML patients had elevated telomerase activity (range 1.7-171.3; mean 42.64±57.65) **(table 2).** In ALL, 23/25 had elevated telomerase activity (range 2.1-149; mean 40.73±39.55) **(table 2).** The level of telomerase activity in acute leukemia patients showed a wide range of distribution. In AML patients, 12 had high/moderate, 9 had low RTA and 4

had normal RTA, while in ALL patients, 16 had high/moderate, 7 had low RTA and 2 had normal RTA. No statistically significant difference was observed in telomerase levels of AML and ALL patients (P >0.091)

Table 2: Statistical comparison of RTA between AML and ALL patients.

	AML (n=25)	ALL (n=25)	Significance
RTA			
Range	1.7-171.3	2.1-149	
Mean	42.64±57.65	40.73±39.55	0.091 (NS)
No of cases			
Normal	4	2	
Low	9	7	
Moderate	7	12	
High	5	4	

RTA = Relative telomerase activity
NS = Non significant (P value >0.05)
Normal < mean + 2SD, Low: above normal-10 RTA, Moderate: 10-50 RTA; High: >50 RTA.

In this study, we compared the hematological findings between patients with various levels of telomerase activity. Our results revealed that in AML and ALL patients, high/moderate telomerase activity levels were associated with high leucocytes count (**AML**: 130.36±78 $X10^3/\mu l$ versus 39.8±314, P = 0.0001; **ALL**: 117.58±119.27 $X10^3/\mu l$ versus 35.37±37.4, P = 0.001) (**table 3**). Also, our results showed no statistically significant difference in BM blast percentages, and telomerase levels in acute leukemia patients (**AML**: P=0.092; **ALL**: P=0.103) (**table 3**).

Table 3: statistical comparison of hematological findings in various levels of telomerase activity in acute leukemia patients.

	AML				ALL			
	L(n=9)	H/M(n=12)	p	Sig.	L(n=7)	H/M(n=16)	p	Sig.
TLC ($X10^3/\mu l$)	39.8±31.4	130.36±78	0.0001	HS	35.37±37.4	117.58±119.27	0.0001	HS
Hb(g//dl)	8.7±1.77	7.8±1.7	0.06	NS	5.78±1.9	6.71±2.65	0.102	NS
Platelets ($X10^5/\mu l$)	44.11±18	53.29±23.6	0.068	NS	54.9±59.9	62.5±73.2	0.4	NS
BM blast (%)	80±13.2	90±5.4	0.092	NS	88.53±3.40	92.25±5.15	0.103	NS

L=Low RTA. H/M= High /moderate RTA. HS= Highly significant

Our study revealed no statistical significant correlation in RTA between different FAB subtypes regarding ALL and AML except that AML-M3 cases (n=3) had low to normal RTA (**fig. 1**).

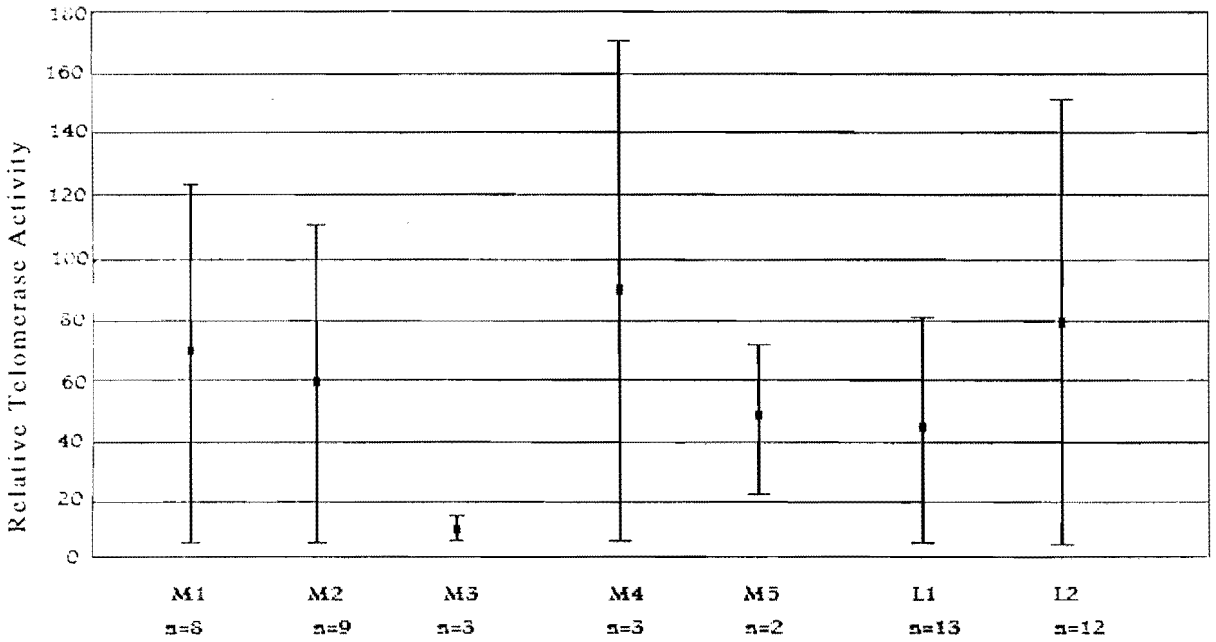

Figure 1: Telomerase activity expressed as relative value compared to FAB classification of AML or ALL diagnosis

DISCUSSION

Human telomerase is a ribonucleoprotein polymerase synthesizes telomere repeat sequences (TTAGGG), at chromosome termini that protect chromosomes from DNA degradation and illegimate recombinations. Both telomerase and telomeres are believed to play important roles in normal and malignant hematopoiesis(10). Telomerase activity is upregulated at least in 80% of acute leukemic cells(17). The biological property of leukemia may depend on cell lineage of leukemia, state of differentiation and clinical stage.

In the present study, we investigated telomerase activity in leukaemic mononuclear blood cells obtained from fifty (50) newly diagnosed acute leukemia patients (25 AML and 25 ALL) and 15, age and sex matched, normal individuals as a control group. As mononuclear cells obtained from acute luekemia patients at diagnosis were composed of more than 80% immature cells. Therefore, the level of telomerase activity measured largely reflects the biological properties of leukaemic cells that were derived from leukaemic stem cells. The reduction of telomerase activity with increasing age have been reported with basal level of telomerase activity is 10-folds higher in children compared to adults(14). For this reason, we examined telomerase activity in normal age-matched peripheral blood mononuclear cells.

In our study, the relative telomerase activity (RTA) was elevated in 21/25 (84%) AML patients with a mean value of 42.64±57.65 RTA and in 23/25 (92%) ALL patients with a mean value of 40.73±39.55 RTA. This is in agreement with recent studies(10,12,20-22) which reported upregulation and over-expression of telomerase activity (TA) in acute leukemia patients. This suggests that telomerase plays an important role in the process of multi-stages leukomogensis and that telomerase activation eventually results in stabilization or elongation of telomeres, subsequent immortalization, increases leukemic potential and disease progression. There was no statistical significant difference in the levels of telomerase activity between AML and ALL patients (P=0.091).

Hiyama et al.(23) reported that the level of telomerase activity increased in haematopoietic progenitor cells upon their proliferation and the committed stem cells rather than the most primitive stem cells had increased telomerase activity. This raised the possibility that