Contents lists available at ScienceDirect

Acta Haematologica Polonica

journal homepage: www.elsevier.com/locate/achaem

Original research article/Praca oryginalna

Telomere length and human telomerase reverse transcriptase (hTERT) level in patients with acute myeloid leukemia: Impact on clinical outcome

*Samia F. El Belbesy, Hanaa A. El Aggan, Hala K. Sultan, Amel A. El Naggar *, Hayat K. Fadlalla Ahmed*

Haematology Department, Medical Research Institute, Alexandria University, Egypt

ARTICLE INFO

Article history:
Received: 02.12.2014
Accepted: 15.07.2015
Available online: 26.07.2015

Keywords:
- Acute myeloid leukemia
- hTERT
- Telomere length
- Response to therapy

ABSTRACT

The response to treatment and overall survival of patients with AML is heterogeneous. Prognostic factors are urgently needed in order to be able to better predict treatment outcomes. The aim of the present work was to study telomere length and human telomerase reverse transcriptase (hTERT) level in acute myeloid leukemia and to detect if these parameters might be useful in providing insight into the clinical outcome of AML patients. ELISA technique was used to measure hTERT level quantitative PCR for measuring telomere length. The study included 70 individuals, 50 patients with acute myeloid leukemia and twenty healthy individuals with comparable age and sex. There was statistically significant higher level of hTERT and lower RTL in patients than controls. The patients were treated according to the standard chemotherapy protocol for induction and they were followed up by bone marrow examination. Mean hTERT level in patients who did not achieve complete hematological remission was statistically significant higher than that in patients who achieved complete hematological remission (48.87 and 34.32 respectively) ($z = -1.98$, $p = 0.048$). Mean RTL in patients who achieved remission was higher than that in patients who did not achieve remission (0.56 and 0.37 respectively). However, it did not reach statistical significance. Median survival time in patients who achieved remission was statistically significant longer than that in those who did not achieve remission (26 ms and 4 ms respectively).

It was found that both relative telomere length and hTERT could be used for assessing clinical behavior and predicting treatment outcome in AML patients.

* *Corresponding author at*: Alferdous Street, Smouha, Alexandria, Egypt. Tel.: +20 01225870627.
E-mail address: amel_elnaggar@hotmail.com (A.A. El Naggar).

http://dx.doi.org/10.1016/j.achaem.2015.07.003

Introduction

Acute myelogenous leukemia (AML) is a malignant disease of the bone marrow in which hematopoietic precursors are arrested in an early stage of development. Despite enormous insights into the molecular mechanisms of acute myeloid leukemia (AML) pathophysiology, this disease is still fatal in the majority of patients, highlighting the urgent need for novel biomarkers useful in AML prognosis and therapy [1, 2].

Telomeres are specialized nucleoprotein structures at the ends of chromosomes; their function is to protect chromosomes from DNA breakage and to prevent chromosome fusion. Without new synthesis, telomeres undergo progressive shortening with each cell division, leading to replicative senescence of cells. Shortening of telomeres can result in telomere end fusions and increase chromosomal instability which is a key initiating event in numerous cancers [3].

Telomerase is an enzyme that extends telomeric repeats on the ends of chromosomes. Activation of telomerase enzyme is therefore required for cells to overcome replicative senescence and to be able to divide indefinitely. Telomerase activity is expressed in germ cells and is present at low level in stem cells, but is usually absent in most somatic cells. Conversely, in immortal cancer cells, telomerase is reactivated, and telomeres are not shortened, suggesting that telomere elongation might be an essential step in tumor formation [4, 5].

Recently genes encoding three major components of human telomerase (TA) have been cloned: human telomerase RNA component (hTR), human telomerase reverse transcriptase (hTERT), and telomerase-associated protein 1 (TAP1). TERT is a telomerase catalytic subunit that is considered as the key component for the control of telomerase activity [6, 7].

Telomere length (TL) is a key determinant of telomere function. Accurate techniques to measure TL in human tissues have provided a greater understanding of the role of telomeres in the progression to malignancy [8].

Targeting the hTERT catalytic subunit as anticancer therapy is theoretically tumor-specific and might be less toxic due to its specific expression in tumor and highly proliferating cells compared to other normal cells. Various newly discovered agents represent interesting anti-hTERT candidates for clinical drug development [9].

The aim of the present study was to study baseline telomere length and human telomerase reverse transcriptase (hTERT) level in acute myeloid leukemia and to detect if these parameters might be useful in predicting response to therapy in acute myeloid leukemia patients.

Subjects

The study included seventy individuals. Fifty newly diagnosed patients with AML and twenty age and sex matched normal healthy individuals as a control group. They were enrolled in the study after the consent of the Ethical Committee of the Medical Research Institute.

The age of AML patients ranged from twenty three to seventy one years with a mean age of 50.8 ± 12.97 years. Twenty-four (48%) patients were males and 26 (52%) were females, with male to female ratio of 0.9.

Patients were treated according to the standard conventional chemotherapy protocol of AML. The treatment protocol entailed the following drugs: Daunorubicin (60 mg/m^2/day for 3 days), and continuous infusion of Cytosine-Arabinoside (100 mg/m^2/day for 7 days) [2]. Patients unfit for receiving the induction cycle were excluded from the study.

Patients were assessed for response to induction therapy at the time of blood count recovery by bone marrow examination. They were divided into two groups: responders who achieved complete hematological remission after induction chemotherapy and non-responders who did not achieve complete hematological remission after induction chemotherapy. The term complete remission generally refers to morphologic complete remission, defined by red blood cell transfusion independence, an absolute neutrophil count of more than 1000/μl and a platelet count of 100 000/μl or greater and the presence of less than 5% blasts in a bone marrow aspirate sample with marrow spicules and with a count of at least 200 nucleated cells, absence of blasts with Auer rods, and absence of extramedullary leukemia [2].

Methods

All subjects participating in this study were subjected to the following:

- Thorough history taking, thorough clinical examination, routine work up including liver and kidney function tests [10], radiological work up (chest X-ray, U/S abdomen &pelvis and ECHO)
- Diagnostic laboratory investigations including, complete blood picture (CBP) [11], bone marrow examination [12], and immunophenotyping [13]
- Quantitative assessment of hTERT by ELISA [14].
  Kit was purchased from GenWay
- quantitative PCR for measuring telomere length [15].
  - Genomic DNA from patients was extracted using QIAamp DNA Blood Mini Kit (50) from Qiagen.
  - Relative telomere length (RTL) were determined using real-time PCR.

Results

The present study included fifty patients with acute myeloid leukemia, and twenty healthy individuals with comparable age and sex (control group).

The age of AML patients ranged from twenty three to seventy one years with a mean age of 50.8 ± 12.97 years. Twenty-four patients were males (48%) and 26 were females (52%), with male to female ratio of 0.9. AML patients included 36 patients aged <60 years (72%), and 14 patients aged ≥ 60 years (28%).

According to the French-American-British (FAB) classification system using morphologic and cytochemical criteria. Thirty-one patients were M2 (62%), 9 patients were M1 (18%), 6 patients were M4 (12%). Other types (M3, M5, M6, and M7) were represented by only one case for each.

Laboratory findings

In AML patients, hemoglobin concentration (Hb) ranged from 4 g/dl to 11.5 g/dl, with mean value of 7.5 ± 2 g/dl. Red blood cell count (RBC) ranged from 1.7 to 4 ($\times 10^{12}$/L), mean value of 2.5 ± 0.6 ($\times 10^{12}$/L). Platelet count (Plt) ranged from 5 to 186 ($\times 10^{9}$/L), with mean value of 34.1 ± 33.4 ($\times 10^{9}$/L). Total leucocytic count (TLC) ranged from 0.3 to 340 ($\times 10^{9}$/L), with mean value of 24.1 ± 50.9 ($\times 10^{9}$/L). Blast % ranged from 0% to 95%, with mean value of 50.5 ± 24%.

Conventional cytogenetic analysis was done for 18 patients only in the present study. Thirteen patients showed normal karyotype. Meanwhile, monosomy 7 was detected in 3 patients, trisomy 21 in one patient, and t(15,17) in one patient.

After induction chemotherapy, patients were followed up by bone marrow examination to determine patient's response to therapy. Complete remission was achieved in nineteen patients (38%), and partial remission in seven patients (14%). Meanwhile, nine patients were resistant to therapy, and fifteen patients died during the first 28 days.

During follow up, nine out of the nineteen patients who achieved CR relapsed.

The mean age of patients who achieved CR after induction chemotherapy (responders) was 45 years. It was statistically significant lower than the mean age of patients who did not achieve CR (nonresponders) which was 54 years ($t = -2.592$, $p = 0.013$).

Serum level of hTERT in AML patients and control subjects

Serum hTERT level in the control group ranged from 2.04 to 11.11 ng/ml, with a mean level of 5.06 ± 2.32 ng/ml. Whereas, serum level in AML patients ranged from 4.9 to 98 ng/ml, with a mean level of 43.3 ± 25.4 ng/ml. There was a statistically significant higher level of hTERT in patients than controls ($z = -6.107$, $p = 0.000$).

The mean serum hTERT level was higher in patients aged ≥60 years than in patients aged <60 years (44 and 43.1 ng/ml respectively), and in females compared to males (43.6 and 42.9 ng/ml respectively). However, this difference did not reach statistical significance.

Relative telomere length by real-time PCR in AML patients and control

Relative telomere length (RTL) in AML patients ranged from 0.01 to 1.1. Mean level was 0.4 ± 0.3. While, in control group, it ranged from 0.85 to 6.77 with mean telomere length of 3.75. RTL was statistically significant lower in patients than control ($z = -6.384$, $p = 0.000$).

The mean RTL was lower in patients aged ≥60 years than in patients aged <60 years (0.4 and 0.5 respectively), and in females compared to males (0.4 and 0.5 respectively). However, this difference did not reach statistical significance.

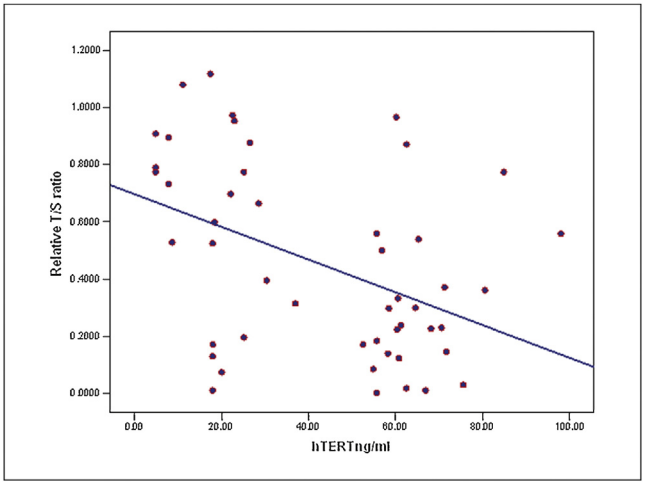

Fig. 1 – Correlation between hTERT and RTL in AML patients ($r = 0.36$, $p = 0.011$). *Interpretation of r_s*: Weak (0.1–0.24); Intermediate (0.25–0.74); Strong (0.75–0.99)

Correlation between hTERT and RTL

There was a negative correlation between serum level of hTERT and the relative telomere length ($p = 0.011$). Level of correlation was intermediate as r_s level was 0.36, as illustrated in Fig. 1.

No statistically significant relation was detected between different FAB subtypes and serum HTERT level or RTL ($p = 0.628$, $p = 0.475$ respectively). However, when FAB subtypes M4 and M5 were grouped together as one group, AML patients with more differentiated subtypes (M4 and M5) had statistically significant shorter telomere length than AML patients with less differentiated subtypes (M1, M2, and M3). Relative telomere length was (0.149 and 0.49 respectively) ($p = 0.011$). But no statistical significant difference between both groups as regard hTERT level.

Serum hTERT level and relative telomere length in relation to response to induction chemotherapy

As demonstrated in Table I, the mean serum hTERT level at diagnosis in patients who did not achieve complete remission (non-responders) was statistically significant higher than that in patients who achieved complete remission

Table I – Comparison of hTERT level and RTL in AML patients according to remission state

Items	Responders	Non-responders	z	p
hTERT (ng/ml)				
• Mean	34.32	48.87	−1.98	0.048
• SD	23.6	25.16		
Relative telomere length				
• Mean	0.56	0.37	−1.59	0.110
• SD	0.36	0.30		

Z: Mann–Whitney test.

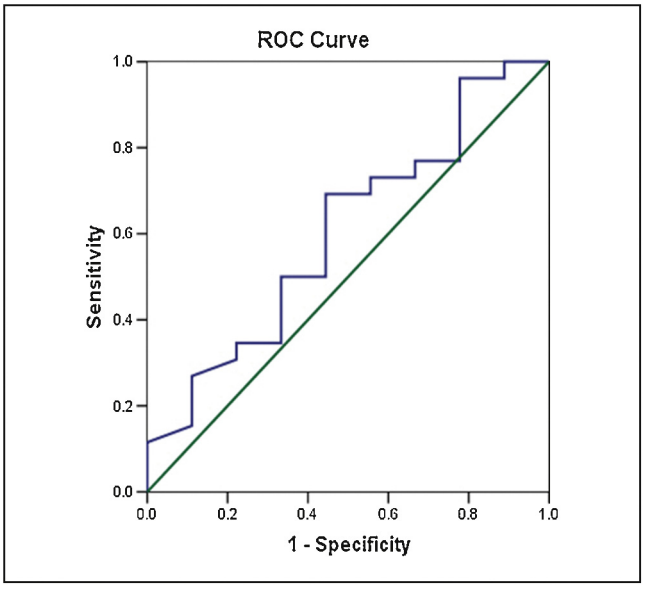

Fig. 2 – ROC curve showing cut off value of 57.57 ng/ml for hTERT as a predictive value for AML outcome

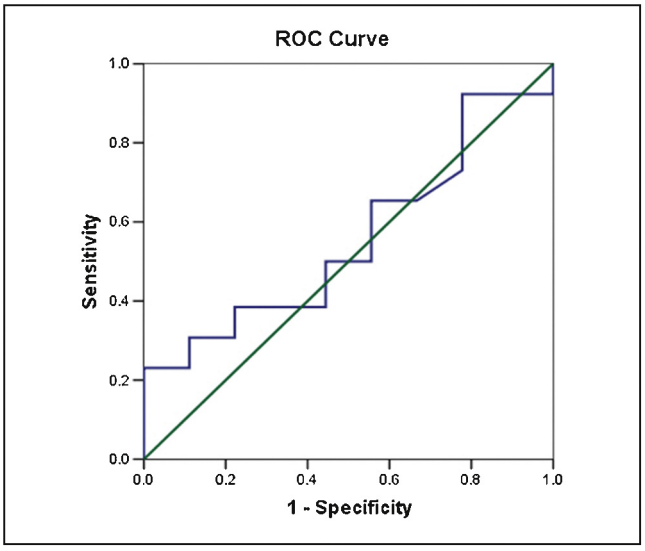

Fig. 3 – ROC curve showing cut off value of 0.5 for RTL as a predictive value for AML outcome

(responders) (48.87 and 34.32 respectively) ($z = -1.98$, $p = 0.048$).

Mean RTL in patients who achieved complete remission (responders) was higher than that in patients who did not achieve complete remission (non-responders) (0.56 and 0.37 respectively). There was no statistical significant difference between both groups ($z = -1.59$, $p = 0.110$).

Using ROC curve, a cut off value of 57.57 ng/ml for baseline serum level of hTERT at diagnosis was calculated which divided the patients into responders and non-responders after receiving the induction chemotherapy. Area under the curve (AUC) = 0.603 (overall accuracy = 60.3%). This means that, in AML patients with value less or equal to 57.57 ng/ml, 60.3% of patients will achieve complete remission (sensitivity was 69.2%, and specificity was 56%).

Similarly, a cut off value of 0.5 was calculated for baseline RTL. AUC = 0.556 (overall accuracy = 55.6%) with sensitivity 50% and specificity 56%. This means that, in AML patients with value equal or more than 0.5, 55.6% of patients will achieve complete remission. As demonstrated in Figs. 2 and 3.

Survival analysis

During duration of our study (30 months), number of events (death) was 36 and censored cases were 14 (total number of our cases = 50).

Using Kaplan–Meier estimate, the median survival time for all AML patients in the study was 10 months (range: 5.271–14.729 months) 95% Confidence Interval.

Median survival time in patients ≥60 years was shorter than that in patients <60 years (8 ms and 10 ms respectively), but there was no statistically significant difference as

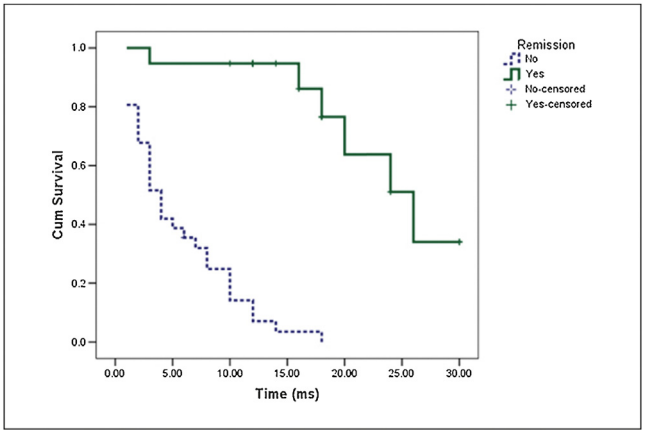

Fig. 4 – Comparison between responders and non-responders regarding survival ($p = 0.001$). *Case Processing Summary*: AML patients in remission: events number = 6, censored number = 13; AML patients not in remission: events number = 30, censored number = 1

regard mean survival time between both age groups (Log Rank (Mantel–Cox) test: Chi-Square = 1.272, $p = 0.259$).

Median survival time was statistically significant higher in patients who achieved complete remission (26 months) compared to those who did not achieve complete remission (4 months) (Log Rank (Mantel–Cox) test: Chi-Square = 38.952, $p = 0.001$). This difference is illustrated in Fig. 4.

Median survival time in relapsed patients was shorter than that in non relapsed patients (20 ms, 26 ms respectively). There was a statistical significant difference in median survival time in both groups (Log Rank (Mantel–Cox) test: Chi-Square = 2.609, $p = 0.050$).

Median survival time in patients with hTERT level higher than cut-off level was shorter than median survival time in

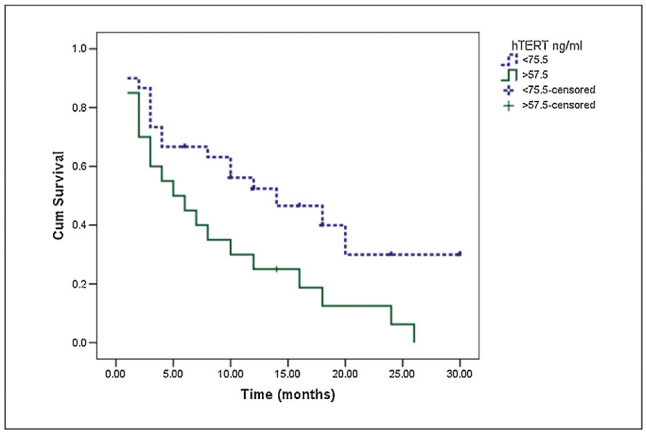

Fig. 5 – Survival comparison according to hTERT level (cut-off level) in AML patients ($p = 0.017$). *Case Processing Summary*: AML patients with higher level than cut-off: events number = 19, censored number = 1; AML patients with lower level than cut-off: events number = 17, censored number = 13

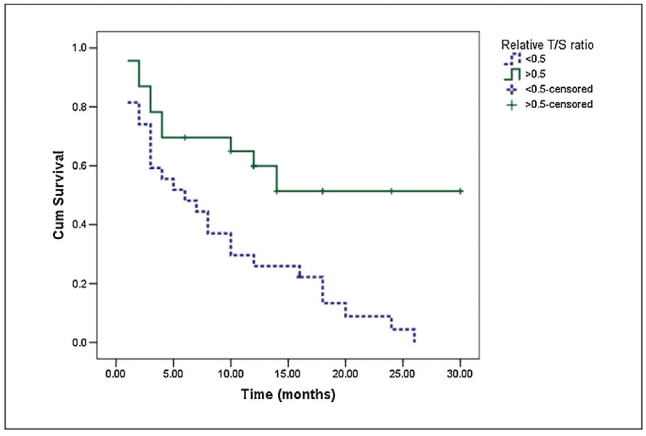

Fig. 6 – Survival comparison according to RTL (cut-off level) in AML patients ($p = 0.002$). *Case Processing Summary*: AML patients with higher RTL than cut-off: events number = 10, censored number = 13; AML patients with lower RTL than cut-off: events number = 26, censored number = 1

patients with lower value (5 ms, and 14 ms respectively). There was a statistical significant difference in median survival time in both groups (Log Rank (Mantel–Cox) test: Chi-Square = 5.653, $p = 0.017$). Similarly, Median survival time in patients with relative telomere length lower than cut-off value was shorter than median survival time in patients with higher value (6 ms, and 14 ms respectively). There was a statistical significant difference in median survival time in both groups. There was a statistical significant difference in median survival time in both groups (Log Rank (Mantel–Cox) test: Chi-Square = 9.305, $p = 0.002$). This is shown in Figs. 5 and 6.

Discussion

The response to treatment and overall survival of patients with AML is heterogeneous. A number of prognostic factors have been described for AML, including age, performance status, and karyotype. These prognostic factors are urgently needed in order to be able to better predict treatment outcomes in defined subgroups of patients [16].

Telomere shortening and telomerase activity has been suggested to be of prognostic value in various human hematopoietic malignancies [17–19].

In the present study, telomere length and human telomerase reverse transcriptase (hTERT) level were measured in acute myeloid leukemia patients to detect if these parameters might be useful in predicting response to therapy in AML patients.

Conventional cytogenetic analysis was done for 18 patients only. Chromosomal abnormalities were detected in 40% of patients (three patients had monosomy 7, one patient had trisomy 21, and one patient had *t*(15,17)) and 59% of AML patients (thirteen patients) had normal karyotype pattern. This finding is in line with the findings of Meng et al. 2013 [20].

The patients were treated according to the standard chemotherapy protocol for induction in AML. They were then assessed for response to induction therapy by bone marrow examination at the time of blood count recovery after chemotherapy. They were divided into two groups: responders, who achieved hematological CR and non-responders who did not achieve hematological CR.

All patients were followed for 30 months (the duration of the study).

Although, several studies have reported telomerase expression in acute and chronic leukemia [17–19]. However, up to our knowledge no large studies have assayed telomerase in quantitative manner.

The present study looked comprehensively into measurement of level of serum hTERT in AML patients and correlated it with treatment outcome. So that, we could understand the prognostic role of telomerase and to predict the efficacy of antitelomerase drugs currently in development.

In the current study, serum hTERT level was significantly higher in AML patients compared to control group. This is consistent with previous studies that reported increased serum hTERT level in several solid tumors as in lung cancer [21, 22], colorectal cancer [23], breast carcinoma [24], laryngeal squamous cell carcinoma [25], in gynecological malignancies [26], in hepatocellular carcinoma [27], and in hematologic neoplasia [28–30]. As most malignant tumors showed increased hTERT activity, it may contribute as part of a multistep process, to human carcinogenesis, as it results in increased telomerase activity. The results of this study is highly supported by the work of Engelhardt et al. [19] who revealed significantly increased telomerase in diagnostic specimens compared with specimens obtained after treatment initiation, which correlated with the disappearance of leukemic cells and with the attainment of remission. In addition, Hartmann et al. in 2005 analyzed hTERT different splicing patterns in AML samples, and

telomerase expression was correlated very well with the expression of the active hTERT splicing variant. Also, Porika et al. 2011 [31] observed that serum hTERT could have a potential application as a novel biomarker for breast cancer diagnosis.

As opposed to the finding in the present work, Greiner et al. [32] reported that, hTERT mRNA was expressed in only 21% of new diagnosed AML patients [32]. Also, Xu et al. [33] showed that hTERT expression was only detectable in AML samples with intermediate or high levels of telomerase activity. This opposing finding could be attributed to different methodologies used. Thus, a direct comparison of the results of various studies is not possible.

In the present study, the mean telomere length in AML patients was significantly shorter, than in healthy control group. This finding is in accordance with previous reports [28, 34, 35]. This is supported by the work of Boultwood et al. [36] who reported that progressive telomere shortening in patients with chronic myelogeneous leukemia in late chronic phase could be linked to up-regulation of telomerase activity and disease evolution in AML [36].

Regarding age, AML patients <60 years had longer telomere length than patients ≥60 years but the difference in telomere length was not statistically significant. This finding is expected as telomeres within hematopoietic cells and other somatic tissues progressively shorten with age [8]. However, Hartmann et al. 2005 [28] found significantly shorter telomere length in the younger group of patients, their finding could be attributed to a higher proportion of karyotypic abnormalities in the younger patients than in the older patients in their population.

A statistically significant negative correlation was found between serum hTERT level and relative telomere length in the present study. This result is in line with the study of Tukun et al. 2006 [37]. This observation could be attributed to the fact that telomerase reactivation is a requirement – if not cause – for unlimited proliferation, which is an essential characteristic of cancer cells. This unlimited dividing capacity provided by telomerase leads to progressive telomere shortening [37].

Serum hTERT and RTL and response to therapy

In the present study, it was observed that serum hTERT level at diagnosis was significantly lower in patients who achieved complete hematological remission than patients who did not achieve complete hematological remission suggesting that hTERT level may act as a predictive marker for the treatment outcome. This finding is supported by the work of Porika et al. 2011 [31] who suggested that pretreatment serum hTERT levels showed a significant correlation with clinical stage in patients with cancer breast.

Relative telomere length at diagnosis was longer in patients who achieved complete remission than in patients who did not achieve complete remission. However, the difference did not reach statistical significance. Further studies with larger number of patients are needed to verify its significance.

Engelhardt [19] observed that, longer telomeres were found after induction chemotherapy in AML and MDS patients, most likely due to the loss of the leukemic clone (with shorter telomeres) and the emergence of normal hematopoietic cells (with longer telomeres) [19].

Serum hTERT level and relative telomere length were compared between patients who survived till the end of the study (total duration of the study was 30 ms) and patients who died during the study. A highly statistical significant difference between both groups as regards mean hTERT level and mean RTL ($p = 0.001$, $p = 0.001$) was found. These observations also suggest that both high hTERT level and short telomere could be considered as poor prognostic factors.

Patients were followed for 30 months (the duration of the study). No statistically significant difference was found between young adult and elderly AML patients in mean survival time using Kaplan–Meier estimate.

This result is not comparable to those obtained in other studies [38, 39] who reported higher incidence of treatment-related mortality in elderly group. However, patients who achieved CR had a statistically significant younger age than patients who did not achieve CR ($t = -2.592$, $p = 0.013$).

These results are in line with studies supporting old age as cause of poor clinical outcome and poor prognostic factor [38, 39].

On the other hand, a high statistically significant difference was detected in median survival time in patients who achieved complete remission compared to those who did not achieve complete remission (26 months and 4 months respectively). The same was observed in relapsed and non relapsed patients as there was a statistically significant difference in median survival time (20 months and 26 months respectively).

Also, survival time was studied in relation to the cut-off value of both hTERT and relative telomere length. A statistically significant longer survival time was observed in patients with hTERT level lower than or equal to the cut-off point and RTL higher than or equal to the cut-off point.

Targeting the hTERT catalytic subunit as anticancer therapy is theoretically tumor-specific and might be less toxic due to its specific expression in tumor and highly proliferating cells compared to other normal cells. Various newly discovered agents represent interesting anti-hTERT candidates for clinical drug development [40, 9].

The telomere and telomerase interactions appear to be an essential determinant for proliferative capacities of tumor cells. It has been known that telomerase activity provides the ability of proliferation to the malignant cell; thus, targeting of tumor cells by inhibiting telomerase may be an effective therapy.

Authors' contributions/Wkład autorów

According to order.

Conflict of interest/Konflikt interesu

None declared.

Financial support/Finansowanie

None declared.

Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

REFERENCES/PIŚMIENNICTWO

[1] Nebbioso A, Benedetti R, Conte M, Iside C, Altucci L. Genetic mutations in epigenetic modifiers as therapeutic targets in acute myeloid leukemia. Expert Opin Ther Targets 2015;30 (May):1–16.
[2] Baer MR, Greer JP. Acute myeloid leukaemia in adults. In: Greer JP, Foerster J, Luken JN, editors. Wintrobe's clinical haematology. 13th ed., Philadelphia, USA: Lippincott William's and Wilkin's publishers; 2013. p. 1843–1889.
[3] Charles H. Telomeres, telomerase, and cancer. N Engl J Med 2000;342:1282–1283.
[4] Klapper W, Krams M, Qian W, Janssen D, Parwaresch R. Telomerase activity in B-cell non-Hodgkin lymphomas is regulated by hTERT transcription and correlated with telomere-binding protein expression but uncoupled from proliferation. Br J Cancer 2003;89:713–719.
[5] Wong JMY, Collins K. Telomerase maintenance and disease. Lancet 2003;362:293.
[6] Bryce LA. Mapping of the gene for the human telomerase reverse transcriptase, hTERT, to chromosome 5p15.33 by fluorescence in situ hybridization. Neoplasia 2000;2: 197–201.
[7] Sabah M, Robert C, Mary L, Elaine K. Immunohistochemical detection of hTERT protein in soft tissue sarcomas: correlation with tumor grade. Appl Immunohistochem Mol Morphol 2006;14(2):198–202.
[8] Baird DM. New developments in telomere length analysis. Exp Gerontol 2005;40:363–368.
[9] Olaussen KA, Dubrana K, Domont J, Spano J, Sabatier L, Charles J. Telomeres and telomerase as targets for anticancer drug development. Crit Rev Oncol Hematol 2006;57(3):191–214.
[10] Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics, 4th ed., St Louis: Elsevier Saunders Company; 2006: 797–825 (renal function tests), 604–614 (liver function tests).
[11] Howven B. The blood cell count. In: Rawan RM, van Assendelft OW, Perston FE, editors. Advanced laboratory methods in haematology. 1st ed., Great Britain: Library of Congress Cataloging; 2002. p. 19–44.
[12] Bates I. Bone marrow biopsy. In: Lewis SM, Bain BJ, Bates I, editors. Dacie and Lewis practical haematology. 9th ed., London: Library of Congress Cataloging; 2001. p. 101–114.
[13] Matutes E, Morilla R, Catovsky D. Immunophenotyping. In: Lewis SM, Bain BJ, Bates I, editors. Dacie and Lewis practical haematology. 9th ed., London: Library of Congress Cataloging; 2001. p. 297–314.
[14] Leffell MS. Immunity and immunological assays. In: Noe DA, Rock RC, editors. Laboratory medicine. 1st ed., USA: Library of Congress Cataloging; 1994. p. 75–97.
[15] Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002;30(10):e47.
[16] Sekeres MA, Peterson B, Dodge RK, et al. Differences in prognostic factors and outcomes in African Americans and whites with acute myeloid leukemia. Blood 2004;103:4036.
[17] Ohyashiki JH, Sashida G, Tauchi T, Ohyashiki K. Telomeres and telomerase in hematologic neoplasia. Oncogene 2002;21:680–687.
[18] Samassekou O, Ntwari A, Hébert J, Yan J. Individual telomere lengths in chronic myeloid leukemia. Neoplasia 2009;11:1146–1154.
[19] Engelhardt M, Mackenzie K, Drullinsky P, Silver R. Telomerase activity and telomere length in acute and chronic leukemia, pre and post-ex vivo culture. Cancer Res 2000;60(13):610–617.
[20] Meng CY, Noor PJ, Ismail A, Ahid MF, Zakaria Z. Cytogenetic profile of de novo acute myeloid leukemia patients in Malaysia. Int J Biomed Sci 2013;9(1):26–32.
[21] White LK, Wright WE, Shay HW. Telomerase inhibitors. Trends Biotechnol 2001;19:114–120.
[22] Peter JFS, Roderick HJB, Thomas GS, et al. Elevated hTERT mRNA levels: a potential determinant of bronchial squamous cell carcinoma (in situ). Int J Cancer 2004;109:412–417.
[23] Liliana T, Enrica R, Salvatore P, et al. Relationship between tumor and plasma levels of hTERT mRNA in patients with colorectal cancer: implications for monitoring of neoplastic disease. Clin Cancer Res 2008;14:7444–7451.
[24] Salhab M, Jiang WG, Newbold RF, et al. The expression of gene transcripts of telomere-associated genes in human breast cancer: correlation with clinico-pathological parameters and clinical outcome. Breast Cancer Res Treat 2008;109:35–46.
[25] Li YR, Wu JM, Wang L, et al. Human telomerase reverse transcriptase expression and its clinical significance in laryngeal squamous cell carcinoma. Acta Otolaryngol 2005;125:409–414.
[26] Miura N, Kanamori Y, Takahashi M, et al. A diagnostic evaluation of serum human telomerase reverse transcriptase mRNA as a novel tumor marker for gynecologic malignancies. Oncol Rep 2007;17:541–548.
[27] Miura N, Maeda Y, Kanbe T, et al. Serum human telomerase reverse transcriptase messenger RNA as a novel tumor marker for hepatocellular carcinoma. Clin Cancer Res 2005;11:3205–3209.
[28] Hartmann U, Brummendorf TH, Balabanov S, Thiede C, Illme T, Schaich M. Telomere length and hTERT expression in patients with acute myeloid leukemia correlates with chromosomal abnormalities. Haematologica 2005;90: 307–316.
[29] Seol JG, Kim ES, Park WH, Jung CW, Kim BK, Lee YY. Telomerase activity in acute myelogenous leukemia: clinical and biological implications. Br J Haematol 1998;100:156–165.
[30] Poole JC, Andrews LG, Tollefsbol TO. Activity, function, and gene regulation of the catalytic subunit of telomerase (hTERT). Gene 2001;269:1–12.
[31] Porika M, Tippani R, Bollam SR, Panuganti SD, Thamidala C, Abbagani S. Serum human telomerase reverse transcriptase: a novel biomarker for breast cancer diagnosis. Int J Clin Oncol 2011;16:617–622.
[32] Greiner J, Ringhoffer M, Taniguchi M, et al. mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the development of specific immunotherapies. Int J Cancer 2004;108:704–711.

[33] Xu D, Gruber A, Peterson C, Pisa P. Telomerase activity and the expression of telomerase components in acute myelogenous leukaemia. Br J Haematol 1998;102: 1367–1375.
[34] Swiggers SJ, Kuijpers MA, de Cort MJ, Beverloo HB, Zijlmans JM. Critically short telomeres in acute myeloid leukemia with loss or gain of parts of chromosomes. Genes Chromosomes Cancer 2006;45:247–256.
[35] Aalbers AM, Calado RT, Young NS, Zwaan CM. Telomere length and telomerase complex mutations in pediatric acute myeloid leukemia. Leukemia 2013;27:1786–1789.
[36] Boultwood J, Peniket A, Watkins F, et al. Telomere length shortening in chronic myelogenous leukemia is associated with reduced time to accelerated phase. Blood 2000;96: 358–361.
[37] Tukun A, Akay G, Kutlay N. Telomere and telomerase in hematological malignancies. Turk J Hematol 2006;23:77–83 [Review article].
[38] Dombret H, Raffoux E, Gardin C. Acute myeloid leukemia in the elderly. Semin Oncol 2008;35:430–438.
[39] Pollyea DA, Kohrt HE, Medeiros BC. Acute myeloid leukaemia in the elderly: a review. Br J Haematol 2011;152:524–542.
[40] Ohyashiki JH, Sashida G, Tauchi T, Ohyashiki K. Telomeres and telomerase in hematologic neoplasia. Oncogene 2002;21(4):680–687.