Egyptian Journal of Medical Human Genetics

RESEARCH Open Access

Telomerase reverse transcriptase gene amplification in hematological malignancies

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Abstract

Background: Telomere is a complex DNA–protein structure located at the end of all eukaryotic chromosomes. The major role of human telomerase is to catalyze the addition of telomeric repeat sequences TTAGGG onto chromosome ends for stabilization of telomere length in attaining cellular immortality and may therefore be a critical step in carcinogenesis. Expression of significant levels of telomerase can dramatically increase proliferative life span and promote cellular immortality, thereby contributing to the malignant phenotype. The purpose of this study is to investigate telomerase reverse transcriptase (TERT) gene amplification in hematological neoplasms, e.g., multiple myeloma (MM), B-non-Hodgkin lymphoma (B-NHL), and acute myeloid leukemia (AML), using FISH technique and to evaluate its potential use as a prognostic marker.

Results: TERT amplification was detected in all groups of the participant patients (15 MM, 15 B-NHL, and 15 AML patients), with higher incidence in AML patients (53.3%). A significant association between the pattern of presentation and telomerase amplification was detected in 88.9% of the relapsed patients who demonstrated amplification of TERT. TERT amplification shows a significant association with p53 deletion and a highly significant association with poor prognosis.

Conclusions: TERT gene amplification is significantly associated with hematological malignancies and may play a critical role in carcinogenesis; thus, elucidation of their regulatory mechanism is highly demanding. Higher amplification was found in relapsed cases than de novo cases which highlight its potential implication in clinical analysis and disease monitoring. Moreover, our results suggest the future use of TERT gene as a potential prognostic marker that may aid in treatment decision and chemotherapy.

Keywords: hTERT gene, Multiple myeloma, Acute myeloid leukemia, B-NHL

Background

Telomeres are unique and genetically stable DNA–protein complex structure located at the ends of all eukaryotic chromosomes to preserve the genome integrity. It contains short non-coding tandem repeats (TTAGGG) that are extended by telomerase enzyme [1].

Telomerase is a ribonucleoprotein enzyme that is formed of two core components: catalytic subunit, human telomerase reverse transcriptase (hTERT), encoded by the TERT gene (located at 5p15.33), telomerase RNA component (TERC) that acts as template for elongation of telomeres [2].

Every cell division is accompanied by shortening of telomere sequences resulting in an "end replication problem" which triggers the activation of DNA damage pathways leading to senescence and subsequent cell death. Telomere erosion and replicative senescence limit cellular proliferation, which is considered an essential tumor-suppressive mechanism [3, 4].

Telomerase expression actively maintains telomere length and hence is crucial for cellular proliferation and survival. Overexpression of telomerase plays an important

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role in the development of cancer, including hematological malignancies [3].

Since hTERT level is believed to be the rate limiting subunit of telomerase so unveiling the mechanisms behind its reactivation is a key step for the development of diagnostic and therapeutic applications [3].

The aim of this work is to investigate TERT gene amplification in hematological neoplasms using FISH technique and to evaluate its potential use as a prognostic marker.

Methods

Study design

This study was conducted on 45 patients recruited from the Internal Medicine Department, between April 2018 and January 2019. The study included 15 multiple myeloma patients, 15 B-non-Hodgkin lymphoma patients, and 15 patients with acute myeloid leukemia (30 male patients and 15 female patients); their age ranged from 38 to 78 years. Patients on treatment were excluded from this study.

The diagnosis of patients was established based on the WHO classification of hematolymphoid tumors [5], and prognosis evaluation was done according to the Revised International Staging System for multiple myeloma [6], International prognostic index of B-NHL [7], and WHO prognostic classification of AML [5].

Clinical examination

All patients were subjected to full history taking; clinical examination with special emphasis on the presence of lymphadenopathy, splenomegaly (SM), and hepatomegaly (HM); signs of anemia and thrombocytopenia; and abdominal ultrasonography for assessing organomegaly.

Sampling

Five milliliters of the venous blood sample was withdrawn from each participant under complete aseptic conditions for complete blood count (CBC) and chemical test analysis. Also, 5 ml bone marrow (BM) aspirate was collected and divided into an EDTA vacutainer for flow cytometric (FCM) analysis and a heparinized vacutainer for cytogenetics investigations.

Laboratory investigations

CBC analysis

CBC was performed on an automatic cell counter Sysmex XS500 (Sysmex, Bohemia, New York, USA) with examination of Leishman-stained smears.

Chemical test

Measurement of serum level of albumin, calcium, and lactate dehydrogenase was performed on automated clinical chemistry analyzer (OLYMPUS AU400), assessment of different fractions of the serum proteins by serum protein electrophoresis.

Immunophenotyping

A flow cytometric immunophenotypic analysis was performed on the BM samples on the same day of their collection, using coulter EPICS-XL four-color flow cytometry (Coulter Diagnostic, Hialeah, FL, USA) using the following panels of monoclonal antibodies (all were supplied by Beckman Coulter, Fullerton, CA, USA).

For the diagnosis of MM: CD19, CD20, CD27, CD28, CD33, CD81, and cyt κ light chain (all are fluorescein isothiocyanate labeled) and CD10, CD13, CD38, CD56, CD117, CD200, and λ light chain (all are labeled by phycoerythrin). Gating was done using phycoerythrin-cyanine-5-labeled CD138 for plasma cells.

For the diagnosis of B-NHLs: CD19, HLA-DR, CD20, CD22, CD23, FMC7, CD103, and κ light chain (all are fluorescein isothiocyanate labeled) and CD5, CD10, CD11C, CD25, CD38, CD52, and λ light chain (all are labeled by phycoerythrin). Gating was done using phycoerythrin-cyanine-5-labeled CD19, and clonality was assessed using κ and λ light chains expressed on CD19+ cells.

For the diagnosis of AML: CD19, CD20, CD33, HLA-DR, CD15, and MPO (all are fluorescein isothiocyanate labeled) and CD34, CD10, TDT, CD38, CD13, CD117, CD5, CD7, CD56, and CD79A (all are labeled by phycoerythrin). Gating was done using phycoerythrin-cyanine-5-labeled CD45.

Cytogenetics investigations

Cytogenetics investigations included:

a) Conventional cytogenetics

Heparinized bone marrow was cultured using complete culture media without phytohemagglutinin for 24 h and 48 h. The cytogenetic preparation and G-banding were done according to routine laboratory procedures according to Verma and Babu [8].

Patients with one or two independent cytogenetic aberrations were regarded as having simple aberrations, whereas those with three or more independent aberrations were regarded as having multiple aberrations.

b) Fluorescence in situ hybridization (FISH) technique: using selected probes LSI hTERT (5p15), p53 (17p13.1) gene, LSI IGH (14q32) break apart rearrangement, and LSI 13q14.3 (Vysis, London, UK)

The heparinized BM samples were cultured using complete culture media without phytohemagglutinin, incubated at 37 °C for 2 days, then harvested as described

previously by Schlette et al. [9]. FISH was carried out according to the manufacturer's instructions, and examination of at least 200 interphase cells was conducted using Cytovision software (Leica, NJ, USA).

Statistical analysis

All of the statistical calculations were made using the excel program and SPSS, version 18 program (SPSS Inc., Chicago, IL, USA). Qualitative data were presented as frequency and percentage, and quantitative parametric data were presented as mean and standard deviation (SD). Association between parameters was done by Pearson chi square. An effect was considered statistically significant at p value less than 0.05 and highly significant at p value less than 0.01.

Results

Demographic, laboratory, and cytogentics features of the studied groups

The study included 45 patients suffering from different hematologic malignancies classified as follows: group I, group II, and group III.

Group I

- This group included 15 MM patients (12 newly diagnosed and 3 relapsed cases), and their age ranged between 55 and 78 years with a mean of 68.2 years. They were 12 males (80%) and 3 females (20%), with a male to female ratio of 4:1.
- Karyotyping of BM was performed for all patients and showed normal karyotype except for one case (6.7%) that had monosomy 13.
- FISH analysis was successfully performed on the 15 BM samples. p53 deletion was detected in 4 patients (26.7%) and 14q32 rearrangement was detected in 3 patients (20%), while 13q14.3 deletion was detected in 5 patients (33.3%).
- TERT gene amplification was detected in 3 patients (20%). The percent of cells with amplification

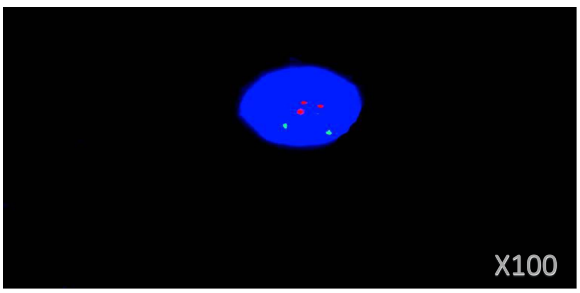

Fig. 1 FISH using hTERT (5p15) spectrum red and control on 5(q)spectrum green show amplification of hTERT (three red signals) in the interphase nucleus from MM patient

Table 1 Descriptive data of multiple myeloma patients (group I)

Parameter	MM patients (N = 15)
Age (mean ± SD)	68.2 ± 6.8
Gender, *N* (%)	
Male	12 (80%)
Female	3 (20%)
Presentation, *N* (%)	
DNV	12 (80%)
Relapse	3 (20%)
Lymphadenopathy, *N* (%)	2 (13.3%)
Splenomegaly, *N* (%)	2 (13.3%)
Hepatomegaly, *N* (%)	1 (6.7%)
WBC count ($\times 10^9$/l) (mean ± SD)	9 ± 5.2
Hb level (g/dl) (mean ± SD)	10.5 ± 2.1
Platelet count ($\times 10^9$/l) (mean ± SD)	146.5 ± 8.5
BM plasma cells% (mean ± SD)	51.6 ± 14
Albumin level, *N* (%)	
< 3.5 g/dl	9 (60%)
≥ 3.5 g/dl	6 (40%)
β2 microglobulin	
< 3.5 mg/l	5 (33.3%)
≥ 3.5 mg/l	10 (66.7%)
Lactate dehydrogenase (U/L) (mean ± SD)	286.7 ± 66
Corrected calcium	
< 10.5 mg/dl	11 (73.3%)
≥ 10.5 mg/dl	4 (26.7%)
Protein electrophoresis, *N* (%)	
Monoclonal gammopathy	6 (40%)
Hypergammaglobulinemia	1 (6.7%)
Hypoalbuminemia	3 (20%)
Normal	5 (33.3%)
Karyotype, *N* (%)	
Normal	14 (93.3%)
Numerical aberrations	1 (6.7%)
P53 (17p13.1) deletion, *N* (%)	4(26.7%)
14q32 rearrangement, *N* (%)	3 (20%)
13q14.3 deletion, *N* (%)	5 (33.3%)
TERT amplification, *N* (%)	3 (20%)
Prognosis, *N* (%)	
I	6 (40%)
II	1 (6.6%)
III	8 (53.3%)

BM bone marrow, *DNV* de novo, *Hb* hemoglobin, *TERT* telomerase reverse transcriptase, *WBCs* white blood cells

ranged from 25 to 60%. The no. of copies ranged from 3 to 5 copies (Fig. 1).

The descriptive and cytogenetics data of MM patients are presented in Tables 1 and 2.

Group II

- This group included 15 B-NHL patients (12 newly diagnosed and 3 relapsed cases), and their age ranged between 52 and 78 years with a mean of 64.3 years. They were 9 males (60%) and 6 females (40%), with a male to female ratio of 1.5:1.
- Karyotyping of BM samples was performed for all patients and showed that 8 cases (53.3%) had normal karyotype, 5 cases (33.3%) had numerical aberration, and 2 cases (13.3%) had structural abnormalities.
- FISH analysis was successfully performed on the 15 BM samples and p53 deletion was detected in 5 patients (33.3%) while 14q32R was detected in 3 patients (20%).
- TERT gene amplification was detected in 7 cases (46.7%). The percent of cells with amplification ranged from 14 to 40%. The no. of copies ranged from 2 to 4 copies.
- The descriptive and cytogenetics data of B-NHL patients are presented in Tables 3 and 4.

Group III

- This group included 15 AML patients (11 newly diagnosed and 4 relapsed cases), and their age ranged between 38 and 68 years with a mean of 47.2 years. They were 9 males (60%) and 6 females (40%), with a male to female ratio of 1.5:1.
- Karyotyping of BM samples was performed for all patients and showed that 8 patients (53.3%) had normal karyotype, 4 patients (26.7%) had numerical aberration, and 3 patients (20%) had structural abnormalities.
- FISH analysis was performed on the 15 BM samples. TERT gene amplification was detected in 8 patients (53.3%). The percent of cells with amplification ranged from 20 to 72%. The no. of copies ranged from 2 to 5 copies (Fig. 2).
- The descriptive and cytogenetics data of AML patients are presented in Tables 5 and 6.

Our results showed that TERT amplification was detected in all groups of the participant patients with higher incidence in AML patients; 8 patients (53.3%) showed amplification, while the least percentage was detected in MM; 3 patients (20%) and 7 patients with B-NHL (46.7%) showed amplification of the TERT.

Regarding the clinical presentation, a significant association was detected between the pattern of presentation and telomerase amplification ($p < 0.05$), 8 cases of the relapsed patients (80%) demonstrate amplification of TERT, while 24 patients with DNV presentation (68.6%) showed no TERT amplification.

In the association analysis presented in Table 7, TERT gene amplification shows a significant association with splenomegaly ($p < 0.05$). However, both hepatomegaly and lymphadenopathy show no significant association with telomerase amplification ($p > 0.05$).

Table 2 Cytogenetics results of multiple myeloma patients (group I)

	Karyotype	P53 del	14q32R	13q14.3 del	TERT ampl % of cells	Range of copy
1	46,xy	30%	Negative	20%	Negative	
2	46,xy	Negative	25%	Negative	Negative	
3	46,xy	Negative	Negative	Negative	Negative	
4	46,xy	Negative	Negative	27%	60%	3–4
5	46,xy	21%	Negative	Negative	Negative	
6	46,xx	Negative	Negative	40%	Negative	
7	46,xy	Negative	Negative	Negative	Negative	
8	45,xx,−13	25%	Negative	30%	25%	4–5
9	46,xy	Negative	Negative	Negative	Negative	
10	46,xy	Negative	Negative	Negative	Negative	
11	46,xx	Negative	32%	Negative	Negative	
12	46,xy	Negative	Negative	20%	Negative	
13	46,xy	30%	Negative	Negative	40%	3–4
14	46,xy	Negative	Negative	Negative	Negative	
15	46,XY	Negative	14%	Negative	Negative	

Table 3 Descriptive data of B-non-Hodgkin lymphoma patients (group II)

Parameter	B-NHL patients (*N* = 15)
Age (mean ± SD)	64.3 ± 7.4
Gender, *N* (%)	
Male	9 (60%)
Female	6 (40%)
Presentation, *N* (%)	
DNV	12 (80%)
Relapse	3 (20%)
Lymphadenopathy, *N* (%)	15 (100%)
Splenomegaly, *N* (%)	10 (66.7%)
Hepatomegaly, *N* (%)	6 (40%)
WBC count ($\times 10^9$/l) (mean ± SD)	13.5 ± 9.5
Hb level (g/dl) (mean ± SD)	11.4 ± 2.9
Platelet count ($\times 10^9$/l) (mean ± SD)	154 ± 65
BM lymphocytes % (mean ± SD)	52.5 ± 20
Lactate dehydrogenase (U/l) (mean ± SD)	277.3 ± 73
Karyotype, *N* (%)	
Normal	8 (53.3%)
Numerical aberrations	5 (33.3%)
Structural aberrations	2 (13.3%)
P53 (17p13.1) deletion, *N* (%)	5 (33.3%)
14q32 rearrangement, *N* (%)	3 (20%)
TERT amplification, *N* (%)	7 (46.7%)
Prognosis, *N* (%)	
I	6 (40%)
II	2 (13.3%)
III	7 (46.7%)

BM bone marrow, *DNV* de novo, *Hb* hemoglobin, *TERT* telomerase reverse transcriptase, *WBCs* white blood cells

As regards the chromosomal aberrations, conventional karyotype analysis revealed no significant association with TERT amplification ($p > 0.05$).

Results by FISH technique show a significant association between p53 deletion and TERT amplification ($p < 0.05$); 6 patients with p53 deletion (66.7%) showed TERT amplification, while only 4 patients (19%) without the deletion had amplification of the TERT.

On the other hand, no association could be detected between both 13q14.3 deletion and 14q32 rearrangement with TERT amplification ($p > 0.05$).

A highly significant association was detected between the bad prognosis and TERT amplification ($p < 0.001$). Twelve patients considered as having bad prognosis had TERT amplification (63.2%), while 16 patients considered as having good prognosis had no TERT amplification (80%).

Finally, no significant association was detected between TERT amplification with age, sex, white blood cell (WBC) count, hemoglobin (Hb) level, and platelet count ($p > 0.05$).

Discussion

Gene amplification is a common genomic disorder in malignant cells which results in an increase of encoded protein synthesis [10]. The association of increased amplification of TERT with the activation of telomerase activity has been anticipated. High telomerase activity is usually observed in germ line cells and most cancer cells, while it is either undetectable or present in very low levels in normal human somatic cells [11].

Increased telomerase activity detected in more than 90% of all cancers correlates with resistance to senescence and apoptosis, immortalization, and elongation of telomere [12]. Therefore, its crucial role in tumor formation has been considered. Furthermore, genetic variants and somatic alterations in the TERT gene may disturb telomerase function and is involved in cancer development as well as the outcome of chemotherapy [3].

In several cases, chromosomal break points were detected nearby the TERT promoter, which suggested that chromosomal rearrangements might be responsible of either relieving the promoter from its suppressive epigenetic environment or placing it close to enhancers at different chromosomal sites [13, 14]. The telomerase reverse transcriptase-cleft lip and palate transmembrane protein 1-like protein (TERT-CLPTM1L) locus containing the gene encoding TERT gene is commonly exposed to somatic chromosomal translocations of immunoglobulin heavy (IGH) and non-IG loci in B cell neoplasms, like in mantle cell lymphoma and splenic marginal zone lymphoma. Additionally, higher TERT transcriptional expression as well as increased telomerase activity were noticed in tumors bearing chromosomal aberrations involving TERT gene [15, 16].

Our results showed that TERT amplification was detected in all studied groups and this is in agreement with several previous studies. In studies done by Nowak et al. [11] and Eid et al. [17], amplification of TERC and TERT genes were detected by FISH technique in 90.4% and 100% (respectively) of AML patients. They suggested that the activation of telomerase in leukemic cells is linked to the amplification of TERC and TERT genes and that the high expression and activity of telomerase spotted in leukemic cells can be clarified by the amplification of these genes. Furthermore, they suggested the possible use of telomerase activity to discriminate malignant from normal cells and that telomerase itself may work as diagnostic marker for tumor development. Moreover, previous studies by Aalbers et al. and Yan et al. [18, 19] reported that genetic alterations causing

Table 4 Cytogenetics results of B-non-Hodgkin lymphoma (group II)

	Karyotype	P53 deletion	14q rearrangement	% of cell TERT amplification	Range of copy number
1	47,XX,+12	Negative	Negative	Negative	
2	46,xx,i(8)(q10)	24%	Negative	Negative	
3	46,xy	Negative	Negative	Negative	
4	46,xx	Negative	Negative	Negative	
5	46,xy	Negative	18%	Negative	
6	47,xy,+5	Negative	Negative	35%	2–3
7	46,xy	Negative	Negative	14%	2–3
8	45,xy,−17	50%	Negative	25%	3–4
9	46,xy	20%	15%	33%	2–3
10	46,xy	Negative	Negative	Negative	
11	46,xx,t(X;11)(q10;q10)	18%	Negative	18%	3–4
12	48,xx,+3,+12	25%	Negative	40%	2–3
13	46,xx	Negative	Negative	Negative	
14	47,xy,+marker	Negative	15%	30%	3–4
15	46,xy	Negative	Negative	Negative	

increased telomerase activity have been implicated in many bone marrow failure syndromes like AML that induced an expansion of undifferentiated myeloid hematopoietic stem cell progenitors.

Several publications reported telomerase activity in B cell malignant lymphomas. In these studies, a positive correlation of telomerase activity with the rate of proliferation in different subtypes of B cell NHLs as well as lymphoid cell lines was observed, suggesting the role of TERT deregulation in lymphomagenesis [20].

A previous study by Xu et al. [21] reported high telomerase activity in MM patients and all cases with plasma cell leukemia. On the other hand, telomerase levels were not elevated in patients with monoclonal gammopathy of undetermined significance (MGUS). However, Panero et al. [20] have found increased expression levels of TERT, in MM as well as in MGUS. Remarkably, in both diseases, the patients displaying the highest telomerase transcription

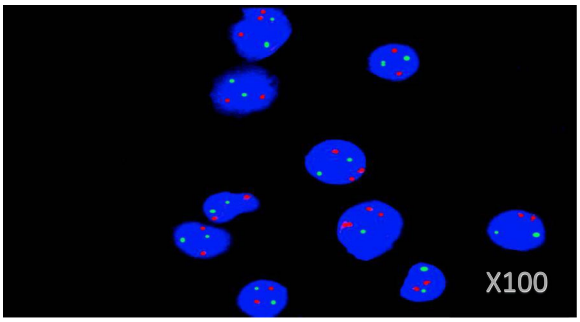

Fig. 2 FISH using hTERT (5p15) spectrum red and control on 5(q)spectrum green show amplification of hTERT (three red signals) in three interphase nuclei from AML patient

Table 5 Descriptive data of acute myeloid leukemia patients (group III)

Parameter	AML patients (*N* = 15)
Age (mean ± SD)	47.2 ± 7.5
Gender, *N* (%)	
Male	9 (60%)
Female	6 (40%)
Presentation, *N* (%)	
DNV	11 (73.3%)
Relapse	4 (26.7%)
Lymphadenopathy, *N* (%)	8 (53.3%)
Splenomegaly, *N* (%)	4 (26.7%)
Hepatomegaly, *N* (%)	3 (20%)
WBC count ($\times 10^9$/l) (mean ± SD)	8.8 ± 5.9
Hb level (g/dl) (mean ± SD)	11.3 ± 2.4
Platelet count ($\times 10^9$/l) (mean ± SD)	110 ± 38
BM blasts % (mean ± SD)	68 ± 21
Karyotype, *N* (%)	
Normal	8 (53.3%)
Numerical aberrations	4 (26.7%)
Structural aberrations	3 (20%)
TERT amplification, *N* (%)	8 (53.3%)
Prognosis, *N* (%)	
Good	8 (53.3%)
Intermediate	3 (20.1%)
Bad	4 (26.6%)

BM bone marrow, *DNV* de novo, *Hb* hemoglobin, *TERT* telomerase reverse transcriptase, *WBCs* white blood cells

Table 6 Cytogenetics results of acute myeloid leukemia (group III)

	Karyotype	% of cell TERT amplification	Range of copy number
1	46,xx	25%	3–4
2	46,xy	Negative	
3	46,xy	30%	3–4
4	47,xy,+8	25%	2–3
5	46,xy	Negative	
6	46,xx,t(8;21)(q22;q22)	Negative	
7	46,xx	Negative	
8	46,xx	33%	2–3
9	46,xy	Negative	
10	45,x,-y	Negative	
11	47,xx,+8	20%	2–3
12	46,xy,t(8;21)(q22;q22)	38%	3–5
13	46,xy,t(15;17)	Negative	
14	46,xx	72%	3–4
15	47,xy,+8	38%	2–3

levels had the shortest telomere length (TL). Diaz de la Guardia et al. [22] demonstrated by gene expression arrays that TERT along with other 16 genes are directly responsible for TL maintenance in MM cells. They even reported higher expression levels of these genes than those in human embryonic stem cells and induced pluripotent stem cells, which have indefinite proliferation capacity [20].

The significant association observed between TERT amplification and bad prognosis is in agreement with previous studies by Röth et al. [23] and Vicente-Dueñas et al. [24] who reported that telomerase activity is not required for the initiation of AML and B cell neoplasm, but it is required for their maintenance. Besides, they stated that enhanced telomerase activity was linked to disease progression, chemotherapy resistance, and worse prognosis in patients with hematologic neoplasia. In addition, telomere shortening could be induced in leukemia cell lines leading eventually to proliferative arrest or cellular apoptosis in vitro and in vivo. The observed functional necessity of telomerase in established hematologic neoplasms offers a basis to therapeutically target telomerase in these diseases [20, 25].

The significant association observed between TERT amplification and p53 deletion is in accordance with Boldrini et al. [26]. They found that altered P53 gene

Table 7 Association between TERT gene amplification and clinicopathological and cytogenetic characteristics of all patients

Variables	TERT amplification negative	TERT amplification positive	*p* value
Age (mean ± SD)	58.6 ± 11.7	61.5 ± 11.7	0.4
Sex			0.52
Male	19	11	
Female	8	7	
Presentation at the time of diagnosis			0.0008
DNV	26	10	
Relapse	1	8	
Hepatomegaly	3	7	0.08
Splenomegaly	4	12	0.004
Lymphadenopathy	12	13	0.3
WBC count (mean ± SD) ($\times 10^3$/μl)	11.03 ± 7.6	9.61 ± 9.8	0.59
Hb level (mean ± SD) (g/dl)	11.39 ± 2.68	10.69 ± 2.3	0.36
Platelet count (mean ± SD) ($\times 10^3$/μl)	147.2 ± 80.4	124 ± 48.5	0.26
Chromosomal aberrations by conventional karyotype	6	9	0.052
13q14.3 deletion	3	2	0.2
14q32 rearrangement	4	2	1
P53(17p13.1) deletion	3	6	0.011
Prognosis			0.021
Good (I)	16	4	
Intermediate (II)	4	2	
Bad (III)	7	12	

DNV de novo, *Hb* hemoglobin, *TERT* telomerase reverse transcriptase, *WBCs* white blood cells

detected in patients with colorectal cancer was significantly associated with both high telomerase activity and increase hTERT expression, suggesting a role of p53 in the signaling pathway for telomerase control. Also, Gonza´lez-Sua´rez et al. [27] suggested the concomitant association between loss of the p53 tumor suppressor and high telomerase activity in inducing tumors during the normal aging process in the mouse.

A previous study done by Rahman et al. [28] reported that the role of p53 in downregulating hTERT may be crucial for the p53-dependent elimination of tumor cells that already express high levels of hTERT. Moreover, p53 inactivation exerted by different mechanisms in tumors may aid in the activation of hTERT and thus contribute to the unlimited replicative potential of tumor cells. Kanaya et al. [29] explained that hTERT could be repressed in tumor cells by p53 through interaction with Sp1 protein or other transcription factors involved in this regulation.

Conclusions

Our data showed that TERT gene amplification is significantly associated with hematological malignancies and may play a critical role in carcinogenesis; thus, elucidation of their regulatory mechanism is highly demanding. Moreover, the highly significant association of TERT amplification and poor prognosis may suggest further use of TERT gene as a potential prognostic marker that may aid in treatment decision and chemotherapy.

Abbreviations

AML: Acute myeloid leukemia; BM: Bone marrow; CBC: Complete blood count; DNV: De novo; FCM: Flow cytometry; FISH: Fluorescence in situ hybridization; Hb: Hemoglobin; HM: Hepatomegaly; hTERT: Human telomerase reverse transcriptase; IGH: Immunoglobulin heavy; LN: Lymph node; MGUS: Monoclonal gammopathy of undetermined significance; MM: Multiple myeloma; NHL: Non-Hodgkin lymphoma; SD: Standard deviation; SM: Splenomegaly; TERC: Telomerase RNA component; TERT-CLPTM1L: Telomerase reverse transcriptase-cleft lip and palate transmembrane protein 1-like protein; TL: Telomere length; WBC: White blood cell

Acknowledgements

We would like to express our gratitude to the National Research Centre and Faculty of Medicine, Ain Shams University, for giving us the chance to accomplish this study with the help of its competent technicians and updated equipment and instrumentation.

Authors' contributions

AH participated in preparing the design of the research, conducting the laboratory work, and preparing the paper for submission and final approval of the version to be published. MT participated in the diagnosis of the patients and conducting the laboratory work. MH participated in performing the laboratory work and preparing the paper for submission. NM participated in performing the laboratory work and statistical analysis. MM participated in preparing the design of the research, conducting the cytogenetics work, and preparing the paper for submission. OM participated in conducting the cytogenetics work and helped to draft the manuscript. RM participated in performing the cytogenetics work and statistical analysis. RY was responsible for the proper selection and diagnosis of the patients. All authors read and approved the final manuscript.

Funding

None.

Availability of data and materials

Data and material are available upon request.

Ethics approval and consent to participate

The study was approved by the ethical committee of the National Research Center (18074), which is in accordance with the ethical standards of the Declaration of Helsinki. All participants gave informed written consent before their inclusion in the study.

Consent for publication

If the paper is accepted by the *Egyptian Journal of Medical Human Genetics*, the study will not be published elsewhere in the same form in English or any other language, and all authors are in agreement with the content of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Received: 3 July 2019 Accepted: 25 October 2019
Published online: 29 November 2019

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