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Telomerase Enzyme Activity in Egyptian Children with Bone Marrow Failure and Response to Immunsupressive Therapy

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ABSTRACT

Background: The development of bone marrow failure might be related to low telomerase activity (TA).

Objectives: We aimed to evaluate TA in children with inherited bone marrow failure (IBMF) and acquired aplastic anemia (AAA). The relation of the acquired disease to TA and response to immunosuppressive therapy (IST) were studied.

Patients and Methods: TA in mononuclear cells was measured utilizing Telomeric Repeat Amplification Protocol (TRAP) in 40 patients and 40 controls.

Results: Median TA was lower in IBMF patients compared to controls (p=0.04), for the AAA cases it was comparable to controls (p=0.228). There was an inverse correlation between TA and age (r=–0.39, p=0.026) but not with disease duration (r=–0.33, p=0.111). Twenty seven AAA patients received Cyclosprine A (CSA); 19 (70.4%) were responderswith median TA of 16.5±4.7 vs. 11.6±3.8 for none responders (p=0.6). Area under the curve (AUC) of TA in predicting IST response among AAA cases was 0.569 (p=0.540).

Conclusion: TA was low in 90% of IBMF and in 23% of severe AAA patients. Evaluation of TA might not be essential for therapeutic or prognostic aspects of AAA. However, it might be useful for selection of stem cell family donors in AAA and IBMF patients with low telomerase activity.

Key Words: *Bone marrow failure – Immune therapy – Telomerase activity – Aquired aplastic anemia.*

INTRODUCTION

The most common cause of bone marrow failure is acquired aplastic anemia (AAA). The inherited bone marrow failure syndromes include Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, and other genetic disorders. Fanconi's anemia and dyskeratosis congenita are the most common types of constitutional aplastic anemia **[1]**. Patients with constitutional aplastic anemia were found to have strikingly short telomeres and low telomerase activity in their cells **[2]**. Telomeres are structural elements that seal the ends of chromosomes, protecting them from recombination, end-to-end fusion, and recognition as damaged DNA. Telomere erosion has been associated with the process of normal aging and defective telomere maintenance is a feature of a variety of human diseases including constitutional aplastic anemia **[3,4]**. Maintenance of the integrity of telomeres requires the telomerase ribonucleoprotein complex **[5-8]**. Most of the acquired aplastic anemia (AAA) is the result of an immune process that destroys hematopoietic stem and progenitor cells **[9,10]**. It was assumed that the predisposition to the development of acquired marrow failure appears to be conferred by genetic alterations resulting in low telomerase activity, short telomeres in leukocytes, and reduced hematopoietic function **[11,12]**. Several studies reported short telomeres and low telomerase activity in leukocytes in up to one third of patients with AAA, especially those who were resistant to immunosuppressive therapy **[12-15]**. Most of the previous studies were concerned mainly with the detection of telomere length and the mutant genes causing low telomerase activity rather than telomerase activity **[12-15]**.

In this study we aimed primarily to evaluate the telomerase functional activity in Egyptian children with inherited bone marrow failure (IBMF) and acquired bone marrow failure namely acquired severe aplastic anemia (AAA).

The relation of the acquired disease to telomerase enzyme activity and response to immunosuppressive therapy were also studied.

PATIENTS AND METHODS

This was a case-control study conducted on unrelated children (n=40) with bone marrow failure syndromes attending the Hematology Clinic during the study period over 3 months from February to April 2014 and forty healthy subjects (age- and sex-matched) taken as controls. The diagnosis of bone marrow failure was based on the presenting clinical features, the blood-count and bone marrow biopsy criteria of the International Agranulocytosis and Aplastic Anemia Study **[16]**. Both groups were enrolled in the study after obtaining consents from their legal guardians and the approval by the Ethical Committee of Cairo University.

Patients included 23 (57.5%) males and 17 (42.5%) females with M/F ratio of 1.35. Mean age of the studied cases was 11.1±4.9 years (range 3.5 to 18, median 11 years). Patients' records were thoroughly reviewed and detailed history-taking was carried out. Disease severity at presentation as well as lines of management and response to immunosuppressive therapy was recorded. Among AAA patients, the disease was considered severe if at least 2 of the following criteria were noted: Neutrophil count less than $0.5x10^9$/L; platelet count less than $20x10^9$/L with hypocellular bone marrow **[17]**.

Immunosuppressive regimens: In AAA patients (n=30); 27 cases received cyclosporine A (CSA) as a monotherapy in a dose range of 7 to 10mg/kg/dto maintain CSA levels between 200-400ng/mL. A combination of anti-thymocyte globulin (ATG) and CSA were given in 3 patients; ATG as a single course for 5 days as intravenous infusion over 12 to 18 hours through a central venous catheter and oral CSA at 5mg/kg/day with the ATG. Oral steroids at a dose of 1mg/kg, prior to each daily dose of ATG to prevent serum sicknesstapered slowly over 4 weeks, was also given. Response to IST was evaluated after initiation of therapy for 3 to 6 months and or follow-up duration ranging from 1 to 13 years with a mean of 5.14±3.84 years.

Evaluation of IST response: Complete response (CR) was defined as a neutrophil count more than $1.5x10^9$/L, a platelet count more than $100x10^9$/L, and a hemoglobin level more than 11.0g/dL **[18]**. Partial response (PR) if neutrophil count was more than $0.5x10^9$/L, platelet count more than $20x10^9$/L, and hemoglobin level was more than 8.0g/dL **[18]**.

Telomerase activity study: Peripheral blood samples were collected from patients and controls under aseptic technique. Mononuclear Cells were separated by Ficoll-Hypaque density gradient centrifugation **[19,20]** and the level of telomerase activity was accurately measured utilizing the Telomeric Repeat Amplification Protocol (TRAP) using the TeloTAGGG Telomerase PCR ELISA^{PLUS} according to Quach et al., **[21]**.

Statistical analysis: Data management and analysis were performed using SigmaStat program; version 3.5 (Systat Software, Inc., USA). The numerical data were statistically presented in terms of range, mean, standard deviation, median and interquartile range (IQR). Categorical data were summarized as percentages. Comparisons between numerical variables of two groups were done by unpaired Student's *t*-test for parametric data or Mann-Whitney Rank Sum test for non-parametric data. Comparing categorical variables were done by Chi-square test or Fisher exact test for small sample size. Pearson product moment correlation test was used for correlating quantitative variables. Receiver's operating characteristics (ROC) curve was made and area under the curve (AUC) was calculated for the telomerase activity in predicting IST response. All *p*-values were two tailed and considered significant when *p*-values less than 0.05.

RESULTS

Demographics and clinical data: The study included 40 patients, 30 cases with severe AAA and 10 with IBMF. Mean age of AAA patients was 10.6±5.0 years (range 3.5 to 18 years, median 9.5yrs) and mean age at diagnosis was 5.2±2.9 (range 1-12, median 5 years). Patients with IBMF included 6 Fanconi anemia (FA), 1 constitutional AA, 1 Dyskeratosis congenita (DKC) and 2 with pure red cell aplasia (PRCA). Their mean age was 14.1±7.1 years (range 4 to 18 years, median 14yrs) and mean age at diagnosis was 6.2±4.3 (range 1.5-12, median 5 years).

Telomerase activity evaluation:

The median telomerase activity was significantly lower in patients with IBMF (p=0.04) while it was insignificantly lower incases with AAA compared to that of controls (Table 1). There was an inverse correlation between the telomerase activity and age (r=–0.39, p=0.026) but no correlation was found between the telomerase activity and disease duration (r=–0.33, p=0.111). Comparing with the mean TA of the control; 90% (9/10) of cases with IBMF had low telomerase activity versus 23% (7/30) of AAA patients (p<0.001).

Table (1): Telomerase activity of the study subgroups and control.

Telomerase level	Median (IQR[a])	Range	p-value
IBMFS[b] (n=10)	5.0 (4.6-8.7)	0.5-45.6	0.043[d]
Control (n=40)	11.2 (5.9-16.6)	1.2-39.0	
AAA[c] (n=30)	5.4 (2.3-21.0)	0.5-65.4	0.228
Control (n=40)	11.2 (5.9-16.6)	1.2-39.0	
IBMFS[b] (n=10)	5.0 (4.6-8.70)	0.5-45.6	0.851
AAA[c] (n=30)	5.4 (2.3-21.0)	0.5-65.4	

[a] IQR : Interquartile range.
[b] IBMFS : Inherited bone marrow failure syndrome.
[c] AAA : Acquired aplastic anemia.
[d] : Statistically significant.

Telomerase activity and response to IST:

All patients with AAA received immunosuppressive therapy for at least 6 months in the form of cyclosporine A (CSA) as a monotherapy (n=27) or combined with ATG (n=3). Nineteen out of the twenty-seven patients (70.4%) were responders to CSA (12 partial responders and 7 complete responders) with no responders among the three cases who received ATG. The median telomerase activity was 16.5±4.7 among AAA responders vs. 11.6±3.8 of none responders but the difference was not significant (p=0.6). Three of the seven cases with low TA responded partially or completely to CSA therapy while 70% (16/23) with normal TA responded to IST (p=0.2). We evaluated the sensitivity and specificity of telomerase activity in predicting the response of AAA patients to IST at different cut off values by ROC curve, we found that area under the curve of telomerase was 0.569 (95% CI 0.377 to 0.748; p=0.540) indicating that the overall predictability of telomerase activity is not significant. On fixing the sensitivity or specificity of telomerase activity, we found that either its sensitivity or specificity became unsatisfactory making its adoption as a good predictor of response in AAA to IST was unlikely (Fig. 1).

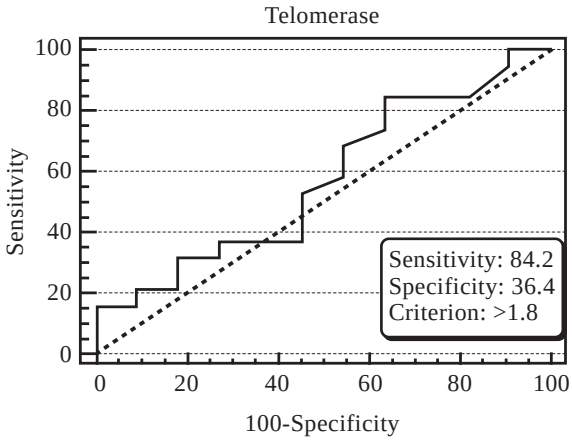

Fig. (1): Receiver Operating (ROC) curve of telomerase activity for prediction of response to Immunesuppressive therapy among acquired aplastic anemia subjects.

DISCUSSION

Our data showed that telomerase activity (TA) was detectable in all of our studied cases including healthy controls. Previous studies reported undetectable TA among healthy controls **[22]**. Children with IBMF had significantly lower TA compared to controls. In AAA patients, the median TA was comparable to IBMF cases; this might be explained by the small number of patients included in this subgroup. In this study up to 23% of cases with AAA had below normal TA which decreased significantly with theincreasing of age, but not related to disease duration. This study showed absence of asignificant direct relationship between TA and response to IST; its adoption as a therapeutic or prognostic predictor was unlikely.

Previous studies evaluated the telomere length and mutations that might affect TA rather than testing for TA directly in patients with acquired marrow failure with short telomeres **[4,11,12,14,23]**. Few authors evaluated TA in patients with AAA who had the TERC or TERT mutations and reported low activity. They found that subjects with low TA did not respond adequately to immunosuppressive therapy. However, their data were mainly descriptive due to the small sample size of patients they studied **[4,12]**.

In IBMF, our data was consistent with the previous studies that reported a low telomerase activity in these patients **[2,24]**. A recent study, which measured telomere length and telomerase activity in 71 aplastic anemia patients, reported that TA had no significant difference in terms of age or gender in the IST responders, non responders or control group. They also found that TA in severe and mild groups was significantly higher than normal control group **[25]**. These data were not in accordance with our data where 23% of our patients with severe AAA had low TA.

Our study had some limitations as we did not test for telomere length and gene mutations in the peripheral leukocytes of those with decreased telomerase activity. However, telomerase activity measurement using the TRAP assay may be an easy and highly sensitive method that may replace mutation and telomere length in the selection of suitable hematopoietic stem cell family donors for transplantation in patients with telomerase deficiency **[21,26]**.

In conclusion, telomerase activity was low in up to 90% of hereditary BMF and in 23% of AAA patients. Evaluation of telomerase activity might not be essential for therapeutic or prognostic aspects of acquired aplastic anemia patients. However, it might be useful for selection of stem cell family donors in patients with acquired aplastic anemia and telomerase deficiency.

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