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# Telomerase enzyme activity in Egyptian children with bone marrow failure and response to immunosuppressive therapy☆

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## 1. 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.

## 2. Materials 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 12 months from June 2013 to May 2014. Forty volunteer healthy subjects (age- and sex-matched) were enrolled and served as a control group. 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 1.4. Mean age of the studied cases was 11.1 ± 4.9 years (range 3.5 to 18 years, 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 were recorded. Among AAA patients, the disease was considered severe if at least 2 of the following criteria were noted: neutrophil count $<0.5 \times 10^9$/L; platelet count $<20 \times 10^9$/L with hypocellular bone marrow [17].

### 2.1. Immunosuppressive regimens

In AAA patients ($n = 30$); 27 cases received cyclosporine A (CSA) as a monotherapy in a dose range of 7 to 10 mg/kg/d to maintain CSA levels

*Abbreviations:* AAA, acquired aplastic anemia; IBMF, inherited bone marrow failure; IST, immunosuppressive therapy; CSA, cyclosporine A; ATG, antithymocyte globulin; CR, complete response; PR, partial response; TA, telomerase activity; TRAP, Telomeric Repeat Amplification Protocol; IQR, interquartile range; ROC, receiver's operating characteristics; FA, Fanconi anemia; DKC, dyskeratosis congenita; PRCA, pure red cell aplasia.

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between 200 and 400 ng/mL. A combination of antithymocyte globulin (ATG) and CSA were given in 3 patients; ATG as a single course for 5 days as intravenous infusion over 12 to 18 h through a central venous catheter and oral CSA at 5 mg/kg/day with the ATG. Oral steroids at a dose of 1 mg/kg, prior to each daily dose of ATG to prevent serum sickness tapered slowly over 4 weeks, was also given. Response to IST was evaluated after initiation of therapy for 3 to 6 months and for follow-up duration ranging from 1 to 13 years with a mean of 5.14 ± 3.84 years.

### 2.2. Evaluation of IST response

Complete response (CR) was defined as a neutrophil count $>1.5 \times 10^9$/L, a platelet count $>100 \times 10^9$/L, and a hemoglobin level >11.0 g/dL [18]. Partial response (PR) if neutrophil count was $>0.5 \times 10^9$/L, platelet count $>20 \times 10^9$/L, and hemoglobin level was >8.0 g/dL [18].

### 2.3. 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].

### 2.4. 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 < 0.05.

## 3. Results

### 3.1. 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 9.5 years) 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 14 years) and mean age at diagnosis was 6.2 ± 4.3 (range 1.5–12, median 5 years).

### 3.2. Telomerase activity evaluation

The median telomerase activity was significantly lower in patients with IBMF ($p = 0.04$) while it was insignificantly lower in cases 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 ($p < 0.001$).

### 3.3. Telomerase activity and clinical variables

A good inverse correlation was detected between the telomerase level and age of the patients ($r = -0.39$, $p = 0.026$). No correlation was found between the telomerase level or disease duration in hereditary or AAA ($r = -0.303$, $p = 0.111$ and $r = 0.305$, $p = 0.156$ respectively).

### 3.4. 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).

## 4. 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 the increasing of age, but not related to disease duration. This study showed absence of a significant 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

**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.