

Survival Analysis of Transfusion Dependent β -Thalassemia Major Patients

Yavarian M. DMT, PhD, **Farsheedfar GR. PhD, *Karimi M. MD, *Almoazzez M. MS, *Harteveld CL. PhD, *** Giordano PC. PhD*

**Haemostasis and Thrombosis Unit, Haematology Research Center, Shiraz University of Medical Sciences, Iran.*

***The Thalassemia Medical Centre, Medical Faculty, Bandar Abbas University, Iran.*

****The Haemoglobinopathies Laboratory, Dept. of Human and Clinical Genetics, Leiden University Medical Center, the Netherlands.*

(Received 5 Apr 2006; accepted in revised form 21 Dec 2006)

Abstract

Background: The classical supportive therapy for β -thalassemia major consists of regular blood transfusion, iron-chelation therapy and specific treatment of the related complications. A balance between the maintenance of the highest possible level of haemoglobin and lowest possible level of iron accumulation in heart and liver give the best chance of survival and the best quality of life.

Methods: For the first time in Iran, we report the survival chance calculated on a cohort of 101 transfusion dependent individuals using Kaplan-Meier analysis. Several factors were included in our study including gender, β -globin genotype, Ferritin level, blood group and access to transfusion, socioeconomic status and type of transfusion protocol.

Results: The survival rate was observed in the first decade of life. While life expectancy up to the age is satisfactory, our study is focused on the decline observed in the second decade of life. Our data shows that 68% of the patients reaches 20 years of age and that prospectively only 50% will still be alive at age 30.

Conclusion: Although information and transfusion protocols play a role, the most important factors influencing the survival rate observed in this study are the ferritin level and the molecular background.

Keywords: *Thalassemia, Survival analysis, Modified Log Rank Test*

Introduction

β -Thalassemia is one of the most common hereditary diseases in man and is highly prevalent in tropical and subtropical regions because of the protection of the carrier against the lethal effects of malaria (1). With rare exceptions, the disease is inherited in an autosomal recessive manner and characterized by partial expression of β -globin in the carrier, and total or almost total absence of expres-

sion in the homozygous or compound heterozygous patient. Conversely, the absence of β -globin expression and the excess of free α globin chains induce the classical severe transfusion dependent clinical phenotype of β -thalassemia major. To reduce the severe symptoms and premature death supportive therapy of thalassemia major have been carefully optimized in the last 3 decades. Regular blood transfusions eliminate severe anaemia, bone marrow expansion and extramedullary erythropoiesis allowing a reasonably normal

Correspondence: Dr Majid Yavarian, Tel: +98 917 361 2059, E-mail: yavarian@sums.ac.ir

development throughout childhood and extend survival (1). However, transfusion therapy results in progressive secondary iron overload with organ and endocrine system damage (2), infection (3) and allo/ autoimmunization risk (4), complications that may result fatal in the second decade of life.

Not all β -thalassemia genotypes result in severe phenotypes. The molecular background can modulate the pathology and be one of the causes which may extend the survival of thalassemia patients (1). Moreover, a low socioeconomic situation and or education may reduce the access to treatment and the outcome of the disease.

The impact of β -thalassemia major is a quite significant issue for the Iranian public health. According to the estimations of the Iranian "Foundation of Special Disease, 1998" at least 20.000 thalassemia patients are living in all Iran with several high prevalence regions and about 1500 of them live in the Hormozgan province, an area which accounts for only 1.7% of the total population in Iran.

We have started this survey due to the mortality increase in the second decade, trying to establish the causes of death, to improve the treatment and to study which factors may contribute to a better patient's care and to a better survival.

Materials and Methods

Population sample

We have followed a cohort of 101 β -thalassemia patients attending the Bandar Abbas thalassemia center, for 5 yr (1999-2005), of whom 50 were females. Since this center takes care of about 90% of the total number of patients in the Hormozgan Province from 1989, the treatment protocols and patient's histories were all available. On average, each patient was transfused monthly and controlled to maintain average Hb levels between 10 and 12 gm/dl. Chelation therapy with desferal was provided either by subcutaneous infusion, intra-

venously or by subcutaneous bolus injection. Most of the patients received subcutaneous infusion in daily doses of 30-50 mg/kg, 5-6 times/wk for the last 5 yr. According to the local facilities (4 blood banks in the region) patients received WBC free blood, washed packed red cell or combinations of both.

Analysis

Haematological analysis was done using standard methods (5). Serum ferritin levels were measured by Immunoradiometric assay (IRMA) (Spectria-Orion Diagnostica, Finland). The molecular characterization of the defects was obtained by DGGE of PCR fragments and by direct sequencing of the β -genes as previously described (6).

Statistical methods

The survival probability was calculated using the Kaplan-Meier methods. The curves obtained by Log Rank Method (7) and Modified Log Rank Methods (8) were compared.

Results

Mortality

During the 3 yr survey, 34 deaths were registered of which, without age correction, 44% were female indicating no significant deference between genders ($P= 0.93$). Equally, no difference between genders was observed on the survival analysis curve when discrimination between urban and rural areas was not applied.

Blood groups and transfusion protocols

On average 70 patients are transfused daily at the thalassemia center in Bandar Abbas. The blood groups distribution among the patients is: O= 41.6%, A= 24.7%, B= 26.7% and AB= 7%. All patients with non-B group received transfusion monthly and maintained the haemoglobin level at the target range of 10 to 12 g/dl. Due to the relatively lower frequency in blood type B (about 20% in the all of the country) and to the fact that blood donors are often not native of the Badar Abbas region, difficulties in finding matching blood

for patient with blood group B may occasionally rise. Therefore some patients with this blood type were less constantly transfused. However, no significant differences in mortality between patients with different blood groups were observed ($P= 0.47$).

No routine matching for secondary blood groups is available up to now in our laboratory. We are however able to detect antibodies against minor blood groups in only 2% of the polytransfused patients after 5 yr of treatment. Of these, positivity for anti c is found in about 70% of the cases. Haemolytic episodes after transfusion are seen on average in about 2-3% of the cases. Only few of them are critical and need specific treatment. The survival probability at the age of 17 calculated according to the blood transfusion regimen shows that patients receiving WBC-free blood have higher probability (75%) than patients receiving washed packed red cells (<60%). Fig. 1 summarize the survival probability expressed at 12 mo intervals showing for the age of 5, 10, 15, 30 and >32, percentages of 99, 93, 85 50 and 30%, respectively.

Ferritin level

As expected, the most significant difference in mortality was observed in association with high serum Ferritin levels ($P= 0.003$). During this survey we have categorized the patients into four groups. Group I with less than 1500 ng/ml; group II, in the range between 1500-2500 ng/ml; group III in the range between 2500-3500 ng/ml and group IV with values higher than 3500 ng/ml. Group III accounted for the highest mortality rate (39.2%). Although 70% of the fewer patients in group IV died during the survey, this group was too small to be used for reliable statistic calculations.

The prediction according to iron load is as follows: At age 12 the chance to survive up to the age of 13 is 93%, 79%, 70% and 19% for the groups I, II, III and IV, respectively. The extrapolation of these data shows that at the age of 24, the chances to get 25 yr old are 79% and 49% for group III and IV, re-

spectively. For group II at the age of 31, the chance to leave one year longer is 30%. Fig. 2-a shows the survival chance at different ages.

Molecular analysis

All 202 independent thalassemia alleles were studied at the molecular level. In total 18 different alleles were found (Table 1). According to Thein (9), these genotypes were classified in three groups: I (β^0/β^0), II (β^0/β^+), and III (β^+/β^+). In absolute values group I accounted for the highest mortality (83%). The expected mortality calculated using both the Log rank test and the modified Log rank test showed comparable probability ($P< 0.004$, Log Rank Test) and ($P< 0.002$, Modified Log Rank test). Fig. 2-b shows that the probabilities to reach the age of 13 at the age of 12 for group I, II, III are 75%, 85% and 90%, respectively. For a patient of group I at the age of 31, the chance of living one year longer will be 30%. The correlation between the probability of survival and genotype I ($P= 0.009$) is strongly significant.

Table 1: Molecular defects found in the 202 β -thalassemia alleles

Allele	Type of mutation	N=202	%
-88, (C→ A)	β^+	1	0,5
Initiation codon, (T→ C)	β^0	2	1,0
Codon 5, -CT	β^0	3	1,5
Codon 8, -AA	β^0	5	2,5
Codon 8/9, +G	β^0	1	0,5
Codon 15, (G A)	β^0	1	0,5
Codon 15, -T	β^0	2	1,0
Codon 30, (G C)	β^0	2	1,0
IVS-I-5, (G C)	β^+	154	76,2
IVS-I-6, (T C)	β^+	1	0,5
Codon 36/37, -T	β^0	2	1,0
Codon 39, (C T)	β^0	3	1,5
Codon 41/42, -TTCT	β^0	3	1,5
Codon 44, -C	β^0	5	2,5
IVS-II-1, (G A)	β^0	12	5,9
IVS-II-745, (C G) In cis 5' UTR +20, C T	β^+	1	0,5
AATAAA AATAAG	β^+	2	1,0
IVS-I 3' end (-25 nts)	β^0	2	1,0

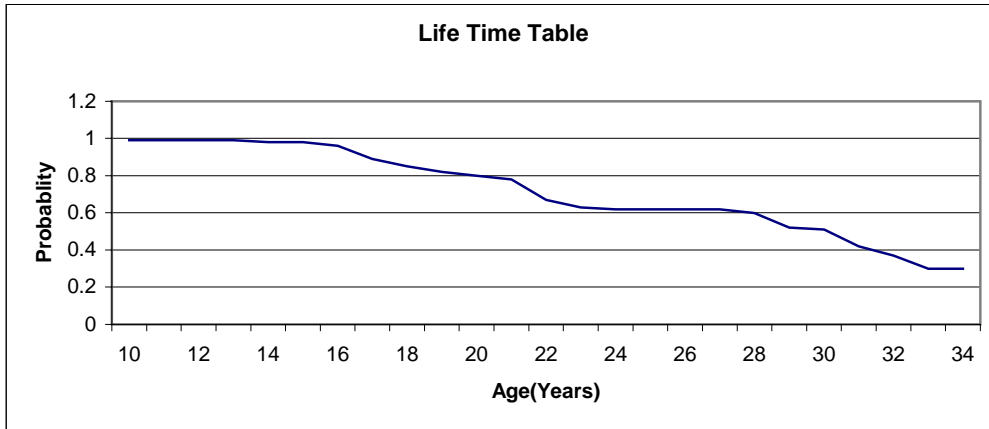


Fig. 1: Life time estimation according to Kaplan-Meier for children with thalassemia in the Hormozgan Province

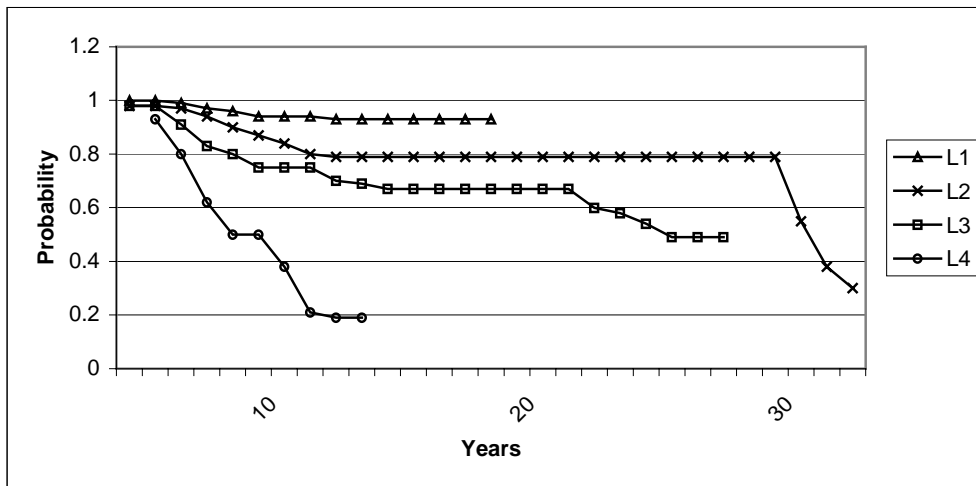


Fig. 2a: Survival curves calculated according to Kaplan-Meier for β -thalassemia patients in the Hormozgan Province. (A) According to serum ferritin levels. L1=less than 1500; L2=1500-2500; L3=2500-3500; L4= more than 3500 ng/ml.

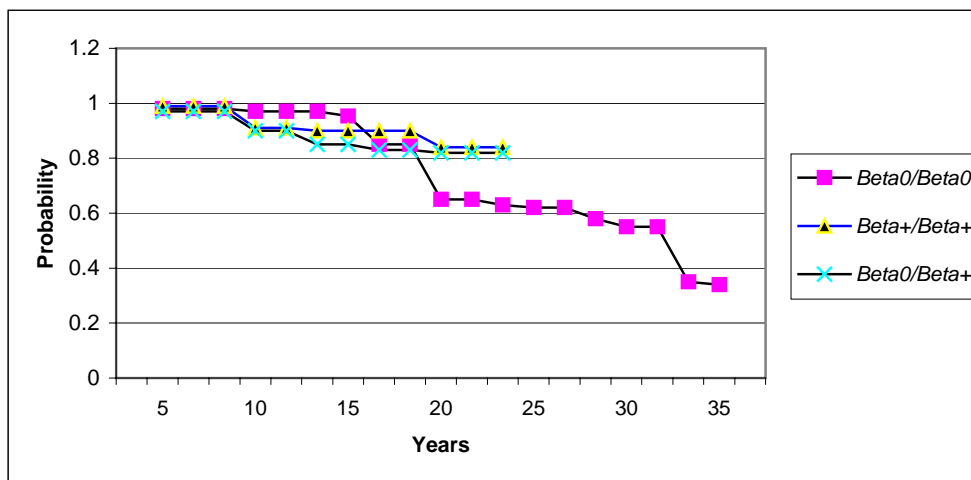


Fig. 2b: Survival curves calculated according to Kaplan-Meier for β -thalassemia patients in the Hormozgan Province according to the β -globin genotype.

Discussion

The "Thalassemia Center of Bandar Abbas", Hormozgan Province, Iran was started in 1989 with the support of the Provincial Thalassemia Association and the local University. From that period on, patient care drastically improved. Infusion pumps for chelation therapy, WBC-free blood became available and clinical protocols drastically improved. This survey is intended to monitor the effect of these improvements and to study, which elements are of major importance in reaching "state of the art" treatment of β -thalassemia in Iran.

During our survey 33.6% of the patients died. Autopsy was not possible due to legal and cultural reason. However, the correlation between mortality and serum Ferritin levels was evident suggesting myocardial iron overload. This according with Ladis et al. (10) who reported that at serum Ferritin levels lower than 2500 $\mu\text{g/l}$ or between 2500-5000 $\mu\text{g/l}$, the chance to survive the age of 30 are 66.9% against 84%, respectively. A similar high mortality rate (70%) was reported in patients with high Ferritin level by Olivieri et al. (11), as the consequence of a cardiac disease in 50% of the cases.

Also our data shows a relatively good survival rate for the well transfused and chelated patients but a very poor especially for the badly chelated one (Fig. 2-a).

Moreover, our survey shows that number medical, cultural and social problems which may bring patients either to a state of unnecessary anaemia or to severe hemosiderosis and to a risk for cardiac failure are still present. An important cultural problem is related to the attitude of some parents in regard to blood transfusion treatment. Some of them realize that blood transfusion will not cure the child and fear that will only generate the need for more transfusions while the child will remain ill. This is obviously true and it is sometime difficult to explain the differ-

ence between a curable and a treatable disease and to explain that is the treatment, which keeps the child alive. Another medical problem related to cultural and social conditions is the poor chelation therapy of some transfused children due to low access or low compliance to subcutaneous infusion of desferal. The adoption of oral chelators (Ferriprox) or a combined treatment could improve the compliance dramatically and allow the access to this essential treatment to a larger share of the patient's population. Moreover, it has been proved that ferriprox in older patients at high risk for fatal cardiomyopathies could open new survival chance for heavily overloaded patients (12-14).

Another medical problem is the still insufficient quality of some blood transfusions. Although all blood transfusions at the thalassemia center of Bandar Abbas are WBC free, the quality of blood products in peripheral centers can still be improved.

We have also observed that, in spite of "free of charge" medical services for thalassemia patients, mortality was about 10% higher in women than in men. This socioeconomic aspect is especially noticed among the 36% of the patients living in rural area.

In conclusion the process toward a better management of β -thalassemia in Iran is still in need of great attention. In comparison with other countries the survival data emerging from our study can still be improved by better transfusion and chelation therapy. Moreover, simple social measures could prevent high early mortality. These measures are based on parent's education, facilitating more effective treatment with regular transfusion chelation therapy (12). Finally, analysis of the molecular defect and genetic background are of great importance to predict the gravity and the evolution of the pathological state of the patients and are the essential "homework" for retrospective and prospective primary prevention.

Of course, being prevention better than cure, information, carrier diagnostics and referral of couples at risk for prenatal diagnosis is offered routinely to couples at risk in the all of the country (6, 15).

Acknowledgements

The authors thank Leiden Medical University for financial supports.

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