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The Effect of Iron Overload in the Hearts of Patients with Beta-Thalassemia

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Summary

Background and hypothesis: An important complication of beta-thalassemia is iron deposition in cardiac tissues resulting in fibrosis and dysfunction. Our aim was the investigation of the possible clinical effect of iron loading in the heart of patients with beta-thalassemia prior to the appearance of symptoms of depressed systolic function.

Methods: Thirty-five patients with beta-thalassemia, of whom 24 had the major type (Group 1) and 11 had the intermedia type (Group 2) were studied. Eleven age- and gender-matched controls were also studied (Group 3). All patients were evaluated echocardiographically and were shown to have normal left ventricular systolic function and dimensions. Serum ferritin, atrial natriuretic peptide (ANP), left atrial diameter (LAD), peak early mitral inflow velocity (E), peak late mitral inflow velocity (A), E/A ratio, deceleration time of the mitral inflow E wave (DT), and isovolumic relaxation time (IVRT) were measured.

Results: Univariate analysis showed that both groups of patients had similarly increased LAD and ANP plasma levels. Group 1 had a higher E/A ratio (2.27 ± 0.88) SS than Group 2 (1.69 ± 0.47, $p = 0.05$) and Group 3 (1.50 ± 0.38, $p = 0.01$). Serum ferritin was significantly higher in Group 1 (3.526 ± 0.352) than in Group 2 (2.808 ± 0.288, $p < 10^{-5}$) and Group 3 (2.139 ± 0.124, $p < 10^{-5}$).Multivariate analysis showed that ANP is a factor that is affected by the LAD and E/A ratio and that serum ferritin levels affect the LAD and E/A ratio.

Conclusions: Although LAD and ANP levels are increased in patients with beta-thalassemia, the increased serum ferritin levels of patients seem to affect left atrial size and E/A ratio. ANP secretion is consecutively affected by these factors.

Key words: beta-thalassemia, atrial natriuretic peptide, ferritin

Introduction

Beta-thalassemia is a hereditary hemolytic anemia due to abnormal synthesis of the chains of adult hemoglobin (HbA). The disease displays considerable variability which is the result of both genetic and acquired factors. Affected individuals are dependent on life-long blood transfusions, the frequency of which is proportional to the severity of the disease.[1,2] Homozygous beta-thalassemia can be classified as major or intermedia depending on the rate of blood transfusions required.[2] Frequent transfusions as required in beta-thalassemia major result in iron overload which affects cardiac structure and function.[3–10] Cardiac dysfunction in this condition is associated with iron deposition in both interstitial cells of the myocardium and ventricular and atrial myocytes[4,5] and usually presents clinically as congestive heart failure after the second decade of life.[6–12] The prognosis is particularly poor.[6,8] Atrial natriuretic peptide (ANP) is a hormone released primarily from atrial myocytes[13–19] during all stages of normal life and in response to various hemodynamic changes.[20–25] Serum ferritin is the most accurate and convenient method for monitoring total body iron load.[26] The aim of this study was to investigate the possible effect of the iron overload in both types of beta-thalassemia in the hearts of patients with normal left ventricular systolic function.

Methods

Thirty-five patients with beta-thalassemia were divided into two groups based on disease severity. Group 1 included 24 patients with beta-thalassemia major who required blood transfusions every 20 days. Patients' mean age ± standard deviation (SD) was 24.54 ± 4.61 years, mean body surface area (±SD)

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was 1.70 ± 0.38 m^2, and the mean (±SD) hemoglobin level prior to blood transfusion was 9.46 ± 0.3 g/dl. Group 2 included 11 patients who had the intermedia type of beta-thalassemia and were rarely transfused. The mean age (±SD) was 26.8 ± 4.4 years, mean body surface area (±SD) was 1.69 ± 0.41 m^2, and mean (±SD) hemoglobin level was 9.38 ± 1.2 g/dl. Eleven healthy age- and gender-matched controls (Group 3) with mean age (±SD) of 27.4 ± 4.2 years, mean body surface area (±SD) 1.75 ± 0.52 m^2, and mean (±SD) hemoglobin level 13.4 ± 0.8 g/dl were also studied.

Patients

All subjects studied were required to have normal left ventricular systolic function and end-diastolic dimensions as assessed by M-mode and two-dimensional (2-D) echocardiography (shortening fraction >25%, ejection fraction >55%, end-diastolic diameter <55 mm, interventricular septum thickness and posterior wall thickness < 1.1 mm). Patients were in sinus rhythm, without any conduction defects. None of the patients or controls was hypertensive. All patients in Group 1 were on chelation therapy, but compliance was variable. Only eight patients in Group 2 were on chelation therapy.

Blood Measurements

Blood sampling for ANP and ferritin measurements was performed just prior to blood transfusion. Patients were transfused with two units of packed red cells. The normal range for serum ferritin in our laboratory is 35 to 200 ng/ml. The mean serum ferritin value in each patient was derived as the mean of 20 values obtained during the preceding 3 years. For ANP measurement, patients were in supine position and 10 ml of blood was collected in plastic tubes containing 10 mg of EDTA and 10,000 kIU of trasylol, such that the final concentration was 1 mg/ml EDTA and 500–1000 kIU/ml trasylol. The specimens were immediately centrifuged at 2000 g and 4°C for 30 min. The plasma was isolated and stored in single-use aliquots at −15°C to −30°C. The following day, samples were purified to extract ANP using Amersham's Amprep 1000 mg C8 columns (code RNP 1902). A three-stage radioimmunoassay technique was then performed and the ANP result was expressed in pg/ml.

Echocardiographic Measurements

An ATL Ultra Mark 9 Ultrasound System was used for the study. M-mode and Doppler echocardiograms were performed in all patients and controls. Patients underwent echocardiogram just before blood measurements and blood transfusion. Left atrial dimension (LAD) was assessed in the long-axis parasternal view using M-mode. The pulsed Doppler signal of mitral inflow was used to measure the following diastolic indices: (1) Isovolumic relaxation time (IVRT): the transducer was positioned at the apical five-chamber view with the sample volume in the left ventricular outflow tract in proximity to the anterior mitral leaflet in order to record both the inflow and outflow signals. The IVRT is the time interval from the end of ejection to the onset of mitral inflow. (2) Peak early (E) velocity and peak late (A) velocity as well as E/A ratio: the transducer was positioned at the apical four-chamber view with the sample volume centered between the mitral leaflets in diastole in order to record maximal inflow velocities. (3) Deceleration time (DT): this was also measured in the apical four-chamber view with the sample volume at the tips of the mitral valve. DT was defined as the time interval from the peak velocity to the end of the E wave. Recordings were obtained at a paper speed of 100 mm/s and included several cardiac cycles to facilitate measurement of velocities and time intervals. The diastolic indices (IVRT and DT) were corrected for the heart rate using the Bajett formula: Corrected time = time measured/R-R interval(s). All measurements were analyzed manually with the incorporated computer digitizing system. Mean values were obtained by averaging at least five cycles. The intraobserver variability of the Doppler measurements was 3.2% and was determined by measuring the same five cycles twice at different times.

Statistics

A first-step univariate analysis was used for intergroup comparisons. To avoid the consequences of multiple comparisons and to control any possible effect between the examined factors, three sets of multiple regression analyses[27] were performed between the three groups of patients (dependent variable); (1) Group 1 versus Group 3, (2) Group 2 versus Group 3, and (3) Group 1 versus Group 2. The same three consecutive models of multiple regression were performed in each set of the groups compared. The rationale for choosing the factors and their order in the models was as follows: The aim of Model 1 was the comparison of echocardiographic variables that were found to be different between groups in the univariate analysis. The investigation of the possible dependence between ANP and echocardiographic variables was the purpose of Model 2 which was formed with the addition of ANP in Model 1. Finally, the addition of ferritin in Model 2 (Model 3) could reveal any possible effect of ferritin in the other variables examined.

Results

The results are shown in Tables I to IV. Table I shows the descriptive statistics of the indicator variables. The Doppler indices DT and IVRT were similar in all groups. However, the E-wave velocity was significantly higher in beta-thalassemia major than in controls, the A-wave velocity showed no statistically significant difference between groups, and the E/A ratio was significantly higher in beta-thalassemmia major than in beta-thalassemia intermedia and controls, but was similar in beta-thalassemia intermedia and controls. Plasma ANP was significantly higher in patients with beta-thalassemia than in controls.

TABLE I Descriptive statistics of the indicator variables of Groups 1, 2, and 3 and intergroup comparisons

Variable	Group 1 (n = 24) Mean ± SD	Group 2 (n = 11) Mean ± SD	Group 3 (n = 11) Mean ± SD	p Value comparison Gr 1 vs. Gr 3	p Value comparison Gr 2 vs. Gr 3	p Value comparison Gr 1 vs. Gr 2
Ferritin (ng/ml)[b]	3.526 ± 0.352	2.808 ± 0.288	2.139 ± 0.124	$<10^{-5}$[a]	$<10^{-5}$[a]	$<10^{-5}$[a]
ANP (pgr/ml)[b]	1.421 ± 0.206	1.489 ± 0.302	1.196 ± 0.274	0.01[a]	0.03[a]	0.44
LAD (mm)	34.83 ± 5.42	33.45 ± 5.43	25.91 ± 6.07	$<10^{-4}$[a]	0.01[a]	0.49
E (m/s)	1.02 ± 0.35	0.82 ± 0.21	0.74 ± 0.15	0.02[a]	0.36	0.09
A (m/s)	0.49 ± 0.25	0.49 ± 0.11	0.51 ± 0.11	0.77	0.69	0.95
E/A ratio	2.27 ± 0.88	1.69 ± 0.47	1.50 ± 0.38	0.01[a]	0.30	0.05[a]
DT (ms)	129.04 ± 34.16	141.73 ± 51.55	125.36 ± 41.47	0.78	0.42	0.39
IVRT (ms)	85.42 ± 22.21	94.55 ± 20.79	96.73 ± 39.71	0.29	0.87	0.26

[a] Statistically significant.
[b] Log 10-transformed.
Abbreviations: GR = group, SD = standard deviation; ANP = atrial natriuretic peptide, LAD left atrial diameter, E = peak early mitral inflow velocity, A = peak late mitral inflow velocity, DT = decelaration time, IVRT = isovolumic relaxation time.

Table II shows the comparison between patients with beta-thalassemia major and controls employing three regression models; these models were fitted to investigate the subset of the indicator variables: log 10-transformed ANP, LAD, E/A ratio, DT, and log 10-transformed ferritin.

In Model 1, the contribution of E/A ratio and LAD were statistically significant (p = 0.03 and p = 0.001, respectively); the additional contribution of log 10-transformed ANP (Model 2) was not significant (p = 0.20), whereas the LAD contribution remained statistically significant (p=0.003) and the E/A ratio became suggestive (p = 0.06); finally, in Model 3, the additional contribution of log 10-transformed ferritin was statistically significant (p = 0.0001), but all the previous differences became statistically not significant.

Table III shows the comparison between patients with beta-thalassemia intermedia and controls employing the same three regression models as in Table II. In Model 1, the LAD variable showed a statistically significant difference (p = 0.02) between these two groups; the additional contribution of log 10-transformed ANP (Model 2) was not significant (p = 0.17), whereas the LAD contribution became suggestive (p = 0.07); finally, in Model 3, the additional contribution of log 10-trans-

TABLE II Simultaneous modeling of three or more variables; comparison between patients with beta-thalassemia major and controls

	Regression coefficient	Standard error	p Value
Model 1			
LAD	0.036	0.009	0.001[b]
E/A ratio	0.173	0.078	0.03[b]
DT	−0.0001	0.002	0.93
Model 2			
Log10 (ANP)	0.353	0.272	0.20
LAD	0.032	0.010	0.003[b]
E/A ratio	0.152	0.079	0.06[a]
DT	0.0001	0.002	0.97
Model 3			
Log10 (ANP)	0.030	0.149	0.84
LAD	0.009	0.006	0.13
E/A ratio	−0.010	0.046	0.83
DT	−0.002	0.001	0.12
Log10 (FER)	0.571	0.065	0.0001[b]

[a] p<0.10.
[b] p<0.05.
Abbreviation: FER = ferritin. Other abbreviations as in Table I.

TABLE III Simultaneous modeling of three or more variables; comparison between patients with beta-thalassemia intermedia and controls

	Regression coefficient	Standard error	p Value
Model 1			
LAD	0.041	0.016	0.02[b]
E/A ratio	0.112	0.240	0.65
DT	−0.0001	0.002	0.97
Model 2			
Log10 (ANP)	0.506	0.352	0.17
LAD	0.032	0.017	0.07[a]
E/A ratio	−0.002	0.246	0.99
DT	0.001	0.002	0.79
Model 3			
Log10 (ANP)	0.280	0.212	0.20
LAD	0.015	0.010	0.15
E/A ratio	0.051	0.146	0.73
DT	0.0002	0.0001	0.86
Log10 (FER)	0.896	0.157	0.0001[b]

[a] p<0.10.
[b] p<0.05.
Abbreviation: FER = ferritin. Other abbreviations as in Table I.

formed ferritin was statistically significant (p = 0.0001), but covered all the previous differences.

Table IV shows the comparison between patients with beta-thalassemia major and intermedia following the same modeling as previously described. In Model 1, the contribution of E/A ratio was suggestive (p = 0.06); the additional contribution of log 10-transformed ANP (Model 2) was not significant (p = 0.17), but the E/A ratio became statistically significant (p = 0.04); finally, in Model 3, the contribution of log 10-transformed ferritin was statistically significant (p = 0.0001), but none of the previous differences was present.

TABLE IV Simultaneous modeling of three or more indicator variables; comparison between patients with beta-thalassemia major and beta-thalassaemia intermedia

	Regression coefficient	Standard error	p Value
Model 1			
LAD	0.011	0.015	0.47
E/A ratio	0.187	0.096	0.06[a]
DT	−0.002	0.002	0.41
Model 2			
Log10 (ANP)	−0.481	0.341	0.17
LAD	0.009	0.014	0.52
E/A ratio	0.211	0.096	0.04[b]
DT	−0.002	0.002	0.25
Model 3			
Log10 (ANP)	−0.206	0.261	0.44
LAD	0.007	0.011	0.54
E/A ratio	0.043	0.079	0.59
DT	−0.003	0.001	0.10
Log10 (FER)	0.677	0.137	0.0001[b]

[a] $p<0.10$.
[b] $p<0.05$.
Abbreviation: FER = ferritin. Other abbreviations as in Table I.

Discussion

Atrial natriuretic peptide is a peptide secreted by atrial myocytes in response to hemodynamic changes in the circulatory system.[20–25] It has a regulatory function interacting with the vascular, neural, and hormonal systems.[15, 28] When developing heart failure, patients with beta-thalassemia have a poor prognosis with a very short life expectancy from 6 months to 1 year.[6, 8] The aim of this study was to investigate whether the iron overload due to transfusions in patients with beta-thalassemia has any effect on the heart early before the symptoms of left ventricular dysfunction appear. In our patients, the echocardiographic and blood measurements were performed just prior to blood transfusion in order to obtain chamber dimensions and ANP values indicative of the basic cardiologic status of the patients without the volume expansion effect of the transfusion.

Our results indicate that beta-thalassemia is associated with significant left atrial distention in comparison with controls (Table I, Model 1 of Tables II and III). The left atrial distention is of a similar degree in patients with beta-thalassemia intermedia and major (Table I, Models 1, 2, 3, Table IV) and may be caused by the hyperkinetic state resulting from chronic anemia.[8] Atrial distention is a stimulus for secretion of ANP.[24, 29, 30]

Serum ferritin levels were higher in Group 1 than in Group 2 (Table I, Model 3 of Table IV), consistent with a greater degree of total body iron overload. Buja and Roberts[5] suggested that iron deposition in the myocardium does not occur until other organs of the hemopoietic system have been saturated. Modest elevations of serum ferritin such as those observed in Group 2 may therefore not be associated with significant iron deposition in the heart.

The indices of left ventricular diastolic function (IVRT and DT) in both groups of beta-thalassemia did not differ significantly from controls (Table I, Models 1, 2, 3 of Tables II, III, IV). The E/A ratio is significantly higher in Group 1 than in Group 3 (Table I, Model 1 of Table II). The mean value of the E/A ratio in this group is >2 (Table I). This finding of E/A > 2 is characteristic of the restrictive ventricular filling pattern seen in congestive heart failure,[31, 32] but its occurrence in patients with normal systolic left ventricular function and without other features of left ventricular diastolic dysfunction may have a different etiology. Kremastinos *et al.*[33] found that adult patients with increased serum ferritin had a mean E/A ratio > 2 which was statistically significantly higher than that in the controls.

The univariate analysis (Table I) showed a statistically significant increase in ANP in both groups of patients. This finding is in accordance with the increased left atrial size of the patients.[24, 29, 30] However, the effect of ANP, although not significantly increased in the echocardiographic parameters, changed the previously observed differences, suggesting that ANP is a confounding factor for the LAD and the E/A ratio (Models 2 of Tables II, III).

A comparison between Groups 2 and 3 showed that ANP is affected by LAD, while such an effect was not found in the corresponding comparison between Groups 1 and 3 (Model 2, Tables II, III). Furthermore, ANP was found to be affected by the E/A ratio (Model 2, Tables II and IV). When the effect of ferritin was examined, all the previously observed differences changed, suggesting that ferritin affects both left atrial dimension and E/A ratio (Models 3, Tables II, III, IV).

The results support the hypothesis of this study, that is, that the higher serum ferritin levels of patients with beta-thalassemia major are associated with greater iron deposition in the heart, resulting in greater damage to myocytes. Dysfunction due to iron deposition may occur earlier in the atria, which are thin-walled chambers, resulting in impaired atrial contractility. We suggest that the high E/A ratio in this clinical context reflects impaired atrial contractility. Similarly, the absence of dependence between LAD and ANP (Model 2 of Table II) may derive from impairment of the cellular synthetic functions of atrial myocytes due to iron overload.

Derchi *et al.*[34] found that patients with normal left ventricular systolic function had increased ANP levels and that some

of these had diastolic dysfunction. In their series, ANP levels were 57% sensitive and 91% specific for detection of left ventricular diastolic dysfunction when compared with echocardiographic measurements. The low sensitivity of ANP for detecting left ventricular diastolic dysfunction when compared with echocardiographic indices suggests that other factors also influence ANP secretion in beta-thalassemia and supports our hypothesis. In our results, the moderate elevation of serum ferritin in beta-thalassemia intermedia was associated with a dependence between LAD and ANP secretion (Model 2, Table III) This degree of iron overload does not appear to compromise the dependence of secretion of ANP in response to atrial distention, but the much higher levels of ferritin seen in beta-thalassemia major are not associated with such a dependence, indicating a possible impaired secretion.

Our results suggest that ANP levels in asymptomatic patients with beta-thalassemia, who do not have evidence of left ventricular diastolic dysfunction, are dependent on left atrial size and E/A ratio. In addition, the dependence of ferritin levels on left atrial size and E/A ratio seems to affect ANP levels. Gennes *et al.*[35] suggested that the cardiac failure in patients with beta-thalassemia is secondary to multiple endocrine abnormalities, which seems to be in agreement with our hypothesis. The not highly increased levels of ANP, as shown from the multivariate analysis (Model 2, Tables II, III), may also explain the very short life expectancy after the onset of symptoms of cardiac failure in this condition.

Study Limitation

Despite the fact that important clinical observations can be made by echocardiography, their confirmation is very difficult. The most accurate method that could prove our hypothesis is the biopsy of the atria from the hearts of patients.

Conclusion

The earliest cardiac manifestations in both types of beta-thalassemia are left atrial distention and statistically increased ANP levels. Serum ferritin levels confirm that iron overload is greater in beta-thalassemia major. Higher iron overload in beta-thalassemia major appears to result in an increased E/A ratio and in the absence of dependence between left atrial distention and ANP levels. Therefore, ANP levels and atrial and ventricular function in beta-thalassemia are complex and ANP does not reliably predict cardiac function in this entity.

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