Neurocognitive dysfunction in children with β thalassemia major: psychometric, neurophysiologic and radiologic evaluation

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ABSTRACT

Objective: To evaluate the impact of iron chelating drugs and serum ferritin on the neurocognitive functions of patients with β thalassemia major (β-TM), using psychometric, neurophysiologic and radiologic tests.

Methods: Eighty children with β-TM were enrolled into the study and were compared to 40 healthy controls. All participants were evaluated by measuring serum ferritin, neurocognitive assessment by Benton Visual Retention Test, Wechsler Intelligence Scale for Children, Wisconsin Card Sort Test, P300 and magnetic resonance spectroscopy (MRS).

Results: WISC in our study showed that 40% of cases were borderline mental function as regards total IQ. Neurophysiologic tests were significantly impaired in patients compared to control group, with significant impairment in those receiving desferrioxamine (DFO). P300 amplitude was significantly lower in cases compared to controls (2.24 and 4.66 uv, respectively), recording the shortest amplitude in patients receiving DFO. Altered metabolic markers in the brain were detected by MRS in the form of reduced N-acetylaspartate to creatine ratio in 78.3% of our cases. There were significant correlations between psychometric tests and both neurophysiologic (P300) and radiologic (MRS) tests.

Conclusion: β-TM is associated with neurocognitive impairment that can be assessed by psychometric, neurophysiologic and radiologic tests. The role of hemosiderosis and iron chelation therapy on cognitive functioning still need more research.

Abbreviations: β-TM: beta thalassemia major; DFO: Dysferal; DFP: Deferiprone; DFX: Deferasirox; WISC: Wechsler Intelligence Scale for Children; VIQ: verbal IQ; PIQ: performance IQ; TIQ: total IQ; BVRT: Benton Visual Retention Test; WCST: Wisconsin Card Sort Test; MRS: Magnetic resonant spectroscopy; NAA/Cr ratio: N-acetylaspartate to creatine ratio

Introduction

Long-term RBC transfusion therapy is required for the treatment of several types of anemia, such as thalassemia. Chronic blood transfusions inevitably lead to iron overload and serious clinical sequelae and patients receiving such transfusions, therefore, require lifelong chelation therapy [1]. There are substantial data demonstrating the efficacy and safety of iron chelation therapy in the treatment of iron overload in regularly transfused patients with β-thalassemia [2,3]. Patients with β-thalassemia major (β-TM) have multiple risk factors for developing central nervous system (CNS) complications. CNS complications generally present as cognitive dysfunction, which usually results from iron deposition and neurotoxicity of desferrioxamine (DFO) which is commonly used as a chelating agent in children with β-TM [4]. Studies reported higher iron deposition in the putamen, caudate nucleus, motor and temporal cortex of patients with β-TM. These areas are as important for cognitive function as for implicit and explicit memory. Other risk factors for brain damage include transient ischemic attacks, asymptomatic brain infarcts and visual and auditory toxicity of DFO [5].

Advances in measuring tissue iron noninvasively by magnetic resonance techniques have enhanced diagnostic capabilities and allowed for more precise measurement and monitoring of iron burden [6].

The major peaks of the proton magnetic resonance spectroscopy (MRS), corresponding to N-acetylaspartate (NAA) and creatine (Cr), have been previously used to evaluate neuronal loss and breakdown of active neurons. Reduction of cerebral N-acetylaspartate to creatine (NAA/Cr) ratio can be used as a dynamic marker of neuronal dysfunction and integrity and cognitive functions [7–9].

The conducted study aims to assess the magnitude of neurocognitive dysfunctions in patients with β-TM and its relation to iron chelating drugs and serum ferritin, using psychometric, neurophysiologic and radiologic evaluation.

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Methods

Eighty children diagnosed with β-TM were on regular blood transfusions to maintain the pre-transfusion hemoglobin concentrations between 7 and 8 g/l especially in the last 3 months. They were recruited from Hematology Outpatient Clinic Ain Shams University. Compliance on treatment defined by self-reports of parent about patient’s adherence to treatment protocol was given [10]. We defined compliance as receiving ≥70% of preplanned iron chelating therapy protocol for 6 months prior to the study. They were divided into four groups according to their iron chelation protocol; group 1 compliant to iron chelating therapy in the form of DFO by subcutaneous infusion (10–12 hours a day) in doses of 20–50 mg/kg body weight/day as home management, group 2 receiving deferiprone (DFP) oral therapy in a dose of 75 mg/kg/day in three divided doses, group 3 on deferasirox (DFX) and group 4 on combined therapy.

They were compared to 40 age- and sex-matched apparently healthy controls (free from any chronic illness, no apparent CNS or genetic disorders that can affect their mental status), coming for routine visits at outpatient clinics.

All children were subjected to detailed history including: age of diagnosis of β-TM, duration of regular blood transfusion, duration of regular chelation therapy, chelation type, compliance and number of blood transfusions per year. A thorough physical examination, including anthropometric measure, full abdominal examination for enlarged liver and/or spleen, was conducted. Other investigations were documented from the patient’s files including: hemoglobin level in the last 3 months and serum ferritin level in the last 3 months. Excluded from the study, children with associated co-morbid condition that can affect their mental state (end-stage renal, hepatic dysfunction, endocrine dysfunction or associated CNS disease and children on medications affecting their mental state).

All the studied cases were subjected to the following tests.

Neuropsychological testing

Wechsler Intelligence Scale for Children third edition

Verbal IQ (VIQ) is based on information, similarities, arithmetic, vocabulary and comprehension. Performance (non-verbal) IQ is based on picture completion, coding, picture arrangement, block design and object assembly. Full-scale IQ is based on 10 tests included in the verbal and performance (non-verbal) IQ scales [11].

Benton Visual Retention Test

Benton Visual Retention Test (BVRT) is designed to assess visual perception, visual memory and visual constructive abilities. Difference between error score and correct score ≥4 signifies neurological impairment [12].

Wisconsin Card Sort Test

Wisconsin Card Sort Test (WCST) is designed to assess the following frontal lobe functions strategic planning, organized searching, utilizing environmental feedback to shift cognitive sets, directing behavior towards achieving a goal and modulating impulsive responding [13].

P300 (P3) or event-related potential

By using the 10–20 system, the reference electrodes were placed over the mastoid regions, while the active electrodes were placed over the Fz and Cz. Electrode resistance was ≤5 kV. The frequency range was between 0.1 and 50 Hz. Thirty-two responses to the target stimuli were averaged. Data were obtained from two consecutive trials. The negative (N) or positive (P) waves were labeled in numerical order. The latency and the amplitude of the P300 (P3) wave were taken into consideration [14,15].

Magnetic resonance spectroscopy

We performed proton MRS single-voxel technique with these parameters short-T1-weighted spin-echo sequence- (TE) 35 milliseconds. We used MRS to measure the concentrations of NAA and Cr in the brain.

The study was approved by our local ethics committee, and informed consent was obtained from all participating families.

Statistical analysis

The collected data were revised, coded, tabulated and introduced to a PC using Statistical Package for Social Sciences (SPSS 15.0.1 for Windows; SPSS, Inc., a division of UNICOM Global, under the UNICOM Intelligence brand., 2001). All numeric variables were expressed as mean – SD. The statistical significance of differences in the baseline characteristics between the groups was evaluated by Student’s test. Chi-square (χ²) test was used to compare frequency of qualitative variables among the different groups. A value of p < 0.05 was considered significant.

Results

In the present study, we have evaluated 80 patients of (6–14 years) mean age 12.08 ± 1.93 years with β-TM who were regularly attending the Pediatric Hematology Outpatient Clinic at Ain Shams University; 51.3% were males, children were compliant on iron chelation protocol for the last 3 months. Thirty-nine patients
(48.8%) were receiving DFP, 32.5% were on combination therapy, 13.8% were on DFO and 5.0% of patients were on DFX; they were compared to 40 healthy individuals of (6–14) mean age (12.23 ± 1.66) years; there were 55% males and 45% females who served as the control group. Patients were receiving average 16.43 ± 4.5 transfusion per year, and the mean age at which transfusion started was 1.07 ± 0.71 years. The serum ferritin was below 1000 ng/ml in 17 (28.3%) of the patients, while 27 (45%) had serum ferritin 1000–2000 ng/ml and 16 (26.7%) with serum ferritin above 2000 ng/ml. Splenectomy was done in 28 (35%) of children with β-TM (Table 1).

Wechsler Intelligence Scale for Children (WISC) in our study showed that 40% of cases were borderline mental function as regards total IQ (TIQ), while 41% were average TIQ, compared to 65% of controls with average TIQ and 22.5% were bright normal. In our study, patient group had significantly lower full-scale (87.56), performance (85.18) and VIQs (92.03) compared with the control group (100.42, 100.50 and 102.80), respectively. The highest VIQ was observed with DFP and DFX (100.42 and 100.50), while highest performance IQ (PIQ) (94.42) and TIQ (97.11) was observed with DFP (Table 2).

BVRT test showed that the difference between obtained error score and expected error score of BVRT was significantly higher in cases compared to controls (Table 2).

Percentage WCST preservative errors were higher in cases compared to controls (20.13 ± 13.54 and 14.50 ± 4.72, respectively). Patients receiving DFO had a significant increase in % preservative error and a decrease in WCST% conceptual level response and WCST categories when compared with other groups (Table 2).

P300 amplitude was significantly lower in cases compared to controls (2.24 and 4.66 uv), respectively, recording the shortest amplitude in patients receiving DFO (Table 3). Also, P300 latency was significantly prolonged in cases compared to controls (386.70 and 316.15 milliseconds, respectively) and recorded the longest duration in patients receiving DFO (Table 3).

In our study, significant negative correlation is observed between P300 latency and most of neuropsychological tests, while significant positive correlation is observed between P300 amplitude and most of neuropsychological tests. Also, there was a significant positive correlation between NAA/Cr and WISC ($r = 0.104$ and $p = 0.000$), and patients with reduced NAA/Cr ratio had a significantly higher abnormal scores on BVRT, with a significant negative correlation between NAA/Cr and BVRT ($r = -0.107$ and $p = 0.002$). Also, WCST scores were significantly lower in patients with reduced NAA/Cr ratio and WCST showed a significant positive correlation with NAA/Cr ratio ($r = 0.448$ and $p = 0.003$) (Table 4).

**Discussion**

Children with β-TM show impaired abstract reasoning, constructional spatial skills and executive functions, which are more prominent in subjects with hemosiderosis [16]. Forty percent of our cases were borderline mental function for TIQ, while 41% were average TIQ;

Table 1. Clinical and demographic data.

|                                | Patients (80) | Controls (40) |
|--------------------------------|---------------|---------------|
| Age (years)                    | 12.08 ± 1.93  | 12.23 ± 1.66  |
| Age at diagnosis of transfusion| 1.05 ± 0.70   | –             |
| Age of first blood transfusion  | 1.07 ± 0.71   | –             |
| Number of transfusion per year  | 16.43 ± 5.40  | –             |
| Weight (kg)                    | 32.24 ± 9.33  | 34.00 ± 5.61  |
| Height (cm)                    | 134.28 ± 14.16| 134.82 ± 6.34 |
| BMI                            | 17.62 ± 2.81  | 18.59 ± 1.83  |
| Sex                            |               |               |
| Male                           | 41 (51.3%)    | 22 (55.0%)    |
| Serum ferritin (<1000) ng/ml   | 17 (28.3%)    | <1000         |
| Serum ferritin (1000–2000)     | 27 (45%)      |               |
| Serum ferritin (>2000) ng/ml   | 16 (26.7%)    |               |
| Compliance to iron             |               |               |
| No                             | 52 (65.0%)    |               |
| Splenectomy                    |               |               |
| Yes                            | 28 (35.0%)    |               |
| DFO: dysferal; DFP: deferiprone; DFX: deferasirox.

Note: BVRT considered normal if the difference between obtained error score and expected error score < 4 and abnormal if ≥ 4, DFO: dysferal; DFP: deferiprone; DFX: deferasirox; VIQ: verbal IQ; PIQ: performance IQ; TIQ: total IQ. There is a significant increase in % preservative error with DFO, while there is a decrease in WCST% conceptual level response and WCST categories with DFO compared with other groups.

$p < 0.05^*$ = significant

Table 2. Psychometric tests (BVRT, WISC and WCST) in children with β-thalassemia on different chelating therapy versus controls.

|                                | DFO N = 11 (%) | DFP N = 39 (%) | DFX N = 4 (%) | Combination N = 26 (%) | Controls N = 40 (%) | p-value |
|--------------------------------|----------------|---------------|--------------|------------------------|---------------------|---------|
| BVRT                           |                |               |              |                        |                     |         |
| Normal (<4)                    | 6 (100)        | 22 (84.6)     | 4 (100)      | 12 (85.7)              | 40 (100)            | 0.294   |
| Abnormal (≥4)                  | 0 (0)          | 6 (15.4)      | 0 (0)        | 2 (14.3)               | 0 (0)               |         |
| Wechsler Intelligence Scale    |                |               |              |                        |                     |         |
| VIQ (mean)                     | 91.83          | 100.42        | 100.50       | 90.00                  | 102.80              | 0.001+  |
| PIQ (mean)                     | 78.83          | 94.42         | 86.75        | 85.57                  | 100.17              | 0.000+  |
| TIQ (mean)                     | 83.83          | 97.11         | 93.25        | 86.64                  | 101.40              | 0.000+  |
| WCST%                          |                |               |              |                        |                     |         |
| Preservative response (mean)    | 45.33          | 15.12         | 12.50        | 23.93                  | 16.27               | 0.009+  |
| Preservative errors (mean)      | 36.67          | 14.19         | 23.00        | 20.79                  | 14.50               | 0.011+  |
| Conceptual level response (mean)| 41.00          | 63.76         | 72.00        | 53.71                  | 68.67               | 0.136   |
| Categories completed (mean)     | 3.00           | 5.11          | 6.00         | 3.86                   | 5.72                | 0.010+  |

Note: BVRT considered normal if the difference between obtained error score and expected error score < 4 and abnormal if ≥ 4, DFO: dysferal; DFP: deferiprone; DFX: deferasirox; VIQ: verbal IQ; PIQ, performance IQ; TIQ, total IQ. There is a significant increase in % preservative error with DFO, while there is a decrease in WCST% conceptual level response and WCST categories with DFO compared with other groups.

$p < 0.05^*$ = significant
this is similar to the study of Economou et al. [17] which reported abnormal TIQ scores (<85) in 36.4% of children with β-TM. Our work echoes similar comparative findings of significantly lower full-scale IQ, performance and VIQs compared with the control group. This is in accordance with the study of Duman et al. [7]. They reported lower scores in the block design subtest of the PIQ and general knowledge and comprehension subtests of VIQ, stating that there is mild cognitive impairment. However, this is in contrast to the study of Logothetis et al. [18] which reported normal intelligence scores in their study group. Economou et al. [17] had 9% of their patients with IQ score under 70 and in another study done, in 1997, by Aydin et al. [19] 80% of their patients had at least one psychiatric disorder and mild mental retardation, borderline intellectual functioning, generalized anxiety disorder, oppositional defiant disorder, major depressive disorder and enuresis.

Our study shows that highest VIQ was observed with DFP and DFX, while highest PIQ as well as TIQ was observed with DFP. It is worth mentioning that the lowest IQ scores were observed with DFO. Similar to the finding of our study, Shehata et al. [20] observed a significant positive correlation between IQ and number of blood transfusion per year. They attributed their finding to washing of toxic agents by frequent blood transfusion and correction of the anemia which lead to significant positive correlation with the studied cognitive functions.

BVRT measures the perception of spatial relations and memory for newly learned material; it is used in the clinical diagnosis of brain damage and dysfunction

in children and adults, as well as in research [8]. However, limited studies have evaluated cognitive function in patients with β-TM and the results have been conflicting. Orsini et al. [21] were the first to report intellectual impairment in such patients. Our study shows abnormal BVRT being significantly higher in cases compared to controls. However, to our knowledge no other studies performed BVRT in β-TM before.

Our study shows that WCST preservative errors were higher in cases compared to controls. WCST measures the ability to learn concepts. It is considered a good measure of frontal lobe functioning. In most cases, neurological involvement does not initially present with relevant signs or symptoms (i.e. subclinical) and can only be detected through neurophysiologic or neuroimaging evaluation [22].

P300 denotes a noninvasive neurophysiologic method of evaluating the CNS; It is used to assess attention, memory discrimination and target detection [23]. Previous research documented delayed P300 latency and lower amplitude of P300 to be accompanied by cognitive impairment [24–26]. Polich et al. [25] reported that increased P300 amplitude was associated with better memory performance. In our study, P300 amplitude was significantly lower in cases compared to controls. This finding is concordant with Shehata et al. [20]. Additionally, our study shows that P300 latency was significantly prolonged in cases compared to controls, which is in contrast to the study done by Duman et al. [7] which did not observe difference in the P300 latency at the Fz and Cz electrode sites in patients and in controls.

### Table 3. Neurophysiologic (P300) and radiologic (MRS) tests in children with β-thalassemia on different chelation therapy versus controls.

| Parameter                  | DFO N = 11 | DFP N = 39 | DFX N = 4 | Combination N = 26 | Controls N = 40 | p-value |
|----------------------------|------------|------------|-----------|--------------------|----------------|---------|
| P300 latency (mean value in milliseconds) | 392.17*   | 371.23     | 384.25    | 387.86             | 316.15         | 0.000*  |
| P300 amplitude (mean value in mV) MRS NAA/Cr ratio Normal >2% | 38.5%     | 15.4%      | 30.8%     | 15.4%              | 100%           | 0.000*  |
| NAA/Cr ratio Reduced <2%  | 31.9%      | 10.6%      | 17%       | 40.4%              | 0%             |         |

DFO: dysferal; DFP: deferiprone; DFX: deferasirox; NAA/Cr ratio: N-acetylaspartate to creatine ratio.
*p < 0.05 considered significant.

### Table 4. Correlations between neurophysiologic, radiologic and psychometric tests.

| Parameter                  | P300 latency (milliseconds) | P300 Amplitude (mV) | MRS (NAA/Cr) |
|----------------------------|-----------------------------|---------------------|--------------|
| P300 latency (milliseconds) | 0.496                       | -0.771              | -0.736       |
| Amplitude (mV)             | -0.356                      | 0.790               | 0.803        |
| MRS (NAA/Cr)              | -0.507                      | 0.625               | 0.739        |

BVRT: Benton Visual Retention Test; WISC: Wechsler Intelligence Scale for Children; WCST: Wisconsin Card Sort Test; MRS: magnetic resonance spectroscopy; NAA/Cr: N-acetylaspartate to creatine ratio.
*p < 0.05 = significant.
p > 0.05 = non-significant.
The relationship between DFO and neurotoxicity needs to be elucidated. Economou et al. [17] defined the therapeutic index as the ratio of mean daily DFO dosage divided by the serum ferritin level and considered it as a risk factor when exceeding 0.025. On the other hand, Porter [27] compared data of DFO pharmacokinetics between asymptomatic β-TM children and those exhibiting severe neurotoxicity, and suggested that DFO-induced neurotoxicity is dose dependent. But Zafeiriou et al. [28] occasionally reported auditory neurotoxicity under long-term DFO treatment.

Reduced NAA/Cr was observed in 78.3% of cases with no significant difference with the type of iron chelation. The relationship between frontal lobe NAA/Cr ratio and neuropsychological tests associated with frontal lobe function was confirmed in a study on healthy children in 2009 by Ozturk et al. [29]. In our study, there was a significant positive correlation between NAA/Cr and WISC, and patients with reduced NAA/Cr ratio had significantly higher abnormal scores on BVRT with a significant negative correlation between NAA/Cr and BVRT. Also, WCST scores were significantly lower in patients with reduced NAA/Cr ratio and WCST showed a significant positive correlation with NAA/Cr ratio.

**Conclusion**

Our findings suggest that β-TM is associated with neuropsychological impairment involving multiple cognitive domains confirmed by different psychometric, neurophysiologic and radiologic tests. However, the relation between iron overload and brain metabolites needs to be further elucidated.

**Limitations of this study** are the presence of some degree of heterogeneity of the studied groups and the external and genetic factors affecting mental stat.

**Acknowledgements**

We thank the children and families participated in this study, also we extend our deepest thanks to our colleagues and professors at Hematology Outpatient Clinic Ain Shams University, who helped us to make this work possible.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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