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ORIGINAL ARTICLE

Polyneuropathy Associated with Severe Iron Overload and Oxidative Stress in β-Thalassemia Patients

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Abstract To investigate the frequency of peripheral neuropathy in patients with β-thalassemia, and to assess its relation to iron overload and oxidative stress. Sixty β-thalassemia patients with mean age of 19 ± 4.9 years were recruited. Serum ferritin was quantitatively assessed by enzyme-linked immunoassay and biomarkers of oxidative stress were estimated calorimetrically. Electrophysiological studies using NEMUS 2, Galileu Software were carried out. The patients were separated into two groups: those with abnormal nerve conduction studies (NCS) {Group A; N = 38} and those with normal NCS {Group B; N = 22}. Thirty-eight (63.3%) patients had axonal motor neuropathy as evidenced by abnormal NCS (group A), they showed higher mean serum ferritin ($p < 0.01$), higher mean malondialdehyde (MDA) ($p < 0.01$), and lower mean nitrous oxide, total antioxidant capacity, paraoxonase-1 (PON1) ($p < 0.01$) compared to group B. Bivariate analysis of NCS data demonstrated that abnormal NCS were more frequent in splenectomized patients ($p = 0.002$), and poorly-chelated patients with serum ferritin ≥ 2000 ng/ml ($p = 0.001$). Significant variables associated with abnormal motor NCS were entered in stepwise regression analysis and only elevated serum ferritin ($p = 0.01$) was independently associated with abnormal motor NCS ($p = 0.02$; 95% CI 1.433–51.791). None of the studied patients had sensory neuropathy or myopathy. Peripheral motor neuropathy may occur in β-thalassemia patients at a high frequency, regardless of their age and gender. Severe iron overload may contribute to the pathogenesis of neuropathy. Other factors including chelation therapy, splenectomy, and oxidative stress might have an enhancing effect that couldn't be proved in this study.

Keywords Polyneuropathy · Iron overload · Oxidative stress-β-Thalassemia

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Introduction

The functional and structural impairment of several organ systems has been documented in β-Thalassemia patients, mainly due to chronic anemia on one hand and to transfusion-related iron overload on the other [1]. Furthermore, dysfunction of the nervous system in β-thalassemia patients has been reported [2] and is usually subclinical and can only be detected during neurophysiological evaluation [3]. Neurological complications have been attributed to various factors such as chronic hypoxia, bone marrow expansion, iron overload, and desferrioxamine neurotoxicity [4]. Recently, oxidative stress was reported as a major

contributor to neural cell injury [5]. In vitro experiments have proved that neural cells cultured with high concentrations of iron die in large quantities because of oxidative stress [6, 7]. In this study, we evaluated the frequency of polyneuropathy in adolescents and young adults with β-thalassemia and investigated whether factors such as age, gender, splenectomy, chelation therapy, oxidative stress, and iron overload are associated with abnormal findings.

Methods

This cross-sectional study included 60 transfusion-dependent β-thalassemia patients aged 12 years or older. They were referred to our hospital, either for blood transfusion or for medical follow-up between December 2015 and May 2016. The study protocol was approved by our Ethics Committee, according to the Institutional Committee for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland. Informed consent was obtained from patients or their legal guardians before enrollment. Patients with anatomical causes of neuropathy, bone marrow expansion, or possible causes of neuropathy like diabetes mellitus or exposure to other known neurotoxic medications, family history of neurological disease, acute febrile illness within 3 weeks prior to enrolment were excluded from the study. All patients were receiving supplemental vitamin B12 and folic acid. None of them was vegetarian or received supplemental antioxidant vitamins (e.g. vitamin E). Data were retrieved by reviewing patients' medical records and direct patients' interviews involving a detailed history-taking. An experienced neurologist performed a thorough clinical examination including testing the cranial nerves, muscle strength, superficial and deep reflexes, and sensations in all four limbs. For grading of muscle strength, we used the Medical Research Council grading system from 0 to 5. Peripheral neuropathy was assessed clinically by Extended Neurologic Evaluation—Total Score *(Total Neuropathy Score, Nurse (TSNn©, Johns Hopkins Univ.);* http://meded.ucsd.edu/clinicalmed/neuro2.htm).

Nerve conduction and electromyographic studies were carried out by the same investigator using *NEMUS 2, Galileu Software*. Measurements were taken at temperatures around 32 °C. Motor nerve conduction studies (NCS) of three motor nerves including the median, ulnar and peroneal nerves were performed. Surface electrode recordings were obtained from the abductor pollicis brevis (on testing the median nerve), the abductor digiti minimi (on testing the ulnar nerve) and the abductor hallucis (on testing peroneal nerve). Sensory NCS of the sural nerve stimulating at the calf and of median or ulnar nerve stimulating at the wrist and elbow were recorded. EMG was done of at least the first dorsal interosseus and anterior tibial muscle on one side. In case of a suspected myopathy, different proximal arm- and leg muscles and also trunk muscles were investigated to detected signs of a myopathy. The patients were separated into two groups: those with abnormal NCS (Group A; N = 38) and those with normal NCS (Group B; N = 22) based on normal values of NCS reported by Albers and colleagues [8].

Eight ml of venous blood were withdrawn, 2 ml were collected on EDTA for complete blood count (CBC) estimation by Coulter Counter. Four ml were collected into a plain tube for assessment of serum ferritin by ELISA technique. The remaining 2 ml were centrifuged for serum separation and frozen at − 80 °C till used for estimation of serum nitric oxide (NO), serum paraoxonase (POX), and serum total antioxidant capacity (TAC) and plasma malondialdehyde (MDA) calorimetrically [9, 10].

Statistical Analysis: The data were analyzed using SPSS (*version 20.0; Chicago, Illinois, USA*) [11]. The Kolmogorov–Smirnov test was used to test the normality of data distribution. Student's t test was used when the distribution of the data was normal, and the values are presented as the mean ± standard deviation (min–max). The Mann–Whitney U test was used for non-normally distributed values. The values of categorically independent groups were compared using the χ^2 test. Univariate correlations between variables were studied using the Spearman's rank-order correlation coefficient. The Stepwise Regression Analysis was done to calculate the WALD, p value and 95% confidence intervals (95% CI) to detect the possible significant risk factors of polyneuropathy. p values < 0.05 were considered to indicate statistical significance.

Results

The studied patients included 22 (36.7%) males, with a male to female ratio of 0.6. All patients were under systematic red blood cell transfusions with a transfusion frequency ranging from 4 to 24 times per year. Forty-nine (81.7%) patients were receiving a type of chelation (Deferiprone 53.3%, Deferoxamine 21.7%, Deferasirox 6.7%), for a duration ranging from 5 to 21 years with a mean duration of 13.24 (± 5.42) years.

Twenty-nine (48.3%) patients suffered from paresthesia and 22 (36.7%) complained of recurrent numbness. Muscle pain was reported during rest in 19 (31.7%), during exercise in 6 (10%) and after exercise in 23 (38.3%) patients. Muscle cramps were reported during rest in 11 (18.3%), during exercise in 3 (5%) and after exercise in 3 (5%) patients (Table 1).

Table 1 Neuromuscular symptoms in the studied patients (n = 60)

	Number	%
Paresthesia		
Yes	29	48.3
No	31	51.7
Numbness		
Yes	22	36.7
No	38	63.3
Muscle pain		
Yes	48	31.7
No	12	68.3
Muscle cramps		
Yes	17	18.3
No	43	81.7
Muscle weakness		
Yes	25	41.7
No	35	58.3

Neurological examination was normal in 25 patients (41.7%) and their extended neurological evaluation score (TNSn) was score 0. Thirty-five patients (58.3%) showed abnormal neurological examination and their TNSn score ranged from 1 to 4. We found positive correlation between Patients' TNSn score and age (r = 0.4, $p < 0.01$), disease duration (r = 0.414, $p < 0.01$), transfusion frequency (r = 0.447, $p < 0.01$) and serum ferritin (r = 0.491, $p < 0.01$).

None of the studied patients had evidence of sensory neuropathy or myopathy while motor NCS revealed that 38 (63.3%) patients had pathological values (Group A). Abnormal findings included a reduction in amplitude the peroneal nerve in 28 (46.7%), reduction in amplitude of the ulnar nerve in 12 (20%) and a reduction in the velocity of the peroneal nerve in 9 (15%) patients. Five of 38 (13%) patients showed abnormal NCS limited to the upper limb. Group A patients had lower amplitudes of the ulnar nerve ($p < 0.01$) and the peroneal nerve ($p < 0.01$) (data not shown). Ulnar and Peroneal nerve conduction amplitudes were found to correlate negatively with age (r = − 0.295, $p < 0.05$; r = − 0.302, < 0.05 respectively).

Group A had higher mean serum ferritin ($p < 0.01$), higher mean malondialdehyde (MDA) ($p < 0.01$), and lower mean nitrous oxide (NO), total antioxidant capacity (TAC), paraoxonase-1 (PON1) ($p < 0.01$) compared to group B (Table 2). Bivariate analysis of NCS data showed that abnormal findings were more frequent in splenectomized patients ($p = 0.002$), and when serum ferritin was ≥ 2000 ng/ml ($p = 0.001$), among those on chelation ($p = 0.01$). Other variables including age and gender did not correlate with the frequency of abnormal findings (Table 3). Significant variables associated with abnormal motor NCS were entered in forwarded stepwise regression analysis and indicated that elevated serum ferritin ($p = 0.01$) was independently associated with abnormal motor NCS ($p = 0.02$; 95% CI 1.433–51.791).

Discussion

Eighty percent of our patients had symptoms and/or signs that could be attributed to neuropathy or myopathy, and the neurophysiological studies (NCS) revealed that 63% patients showed motor neuropathy of upper and/or lower limbs in the absence of sensory neuropathy or myopathy. A striking finding was the isolated affection of the upper limbs in 5 out of 38 patients. Our data showed that Median nerve was spared and its values were within normal range in all patients, while the mean amplitude values of Ulnar and Peroneal nerves were significantly lower in symptomatizing patients when compared to patients with normal NCS and this was in agreement with previous studies [2]. Muscle weakness and muscle pain which were reported by many of our patients might be due to false interpretation of joint pain or tiredness. But possible effects of iron on muscle metabolism leading to these complaints couldn't be excluded.

In fact, little thought was given to concern about peripheral neuropathy and NCS in β-thalassemia patients. Myopathy syndrome in patients with β-thalassemia major was first described in an old study that reported more involvement of the lower limbs [12]. In 1991, Papanastasiou et al. [14] reported that 22% of their study population had mild peripheral neuropathy, mainly motor, during the second decade of life. A few years later, a second study reported clinically evident neuropathy with decreased motor NCV in 10% of their patients [13]. A more recent study reported that 25% of patients with β-thalassemia had neurological symptoms, 22% had neurological signs of neuropathy, and 53% had pathological values in the NCS [2]. Another study examined 30 patients with β-thalassemia and reported that 78% of them had mild sensory polyneuropathy [15].

Chelation is an integral part of treatment in β-thalassemia patients that might play a role in peripheral neuropathy. Previous reports proved that deferoxaminne is neurotoxic especially when used at high doses and for a long duration [16]. However, when the literature was reviewed, we found no standardized method applied to assess DFO-related neurotoxicity, which may give rise to conflicting results. In the case of deferasirox and deferiprone, ototoxicity was found to occur in less than 1% of patients. Furthermore, all patients on iron chelation are already suffering from hemosiderosis which might be the

Table 2 Comparison of laboratory variables between group A and group B

Variables	Group A (n = 38) Mean ± SD	Group B (n = 22) Mean ± SD	*p* value
Hb (g/dl)	6.92 ± 0.96	7.21 ± 1.29	0.391
Ferritin (ng/ml)	3455.89 ± 296,016	1353.40 ± 1067.19	< 0.01*
NO (um/ml)	14.34 ± 5.86	16.8 ± 5.3	< 0.01*
MDA (nmol/ml)	4.53 ± 1.62	0.7 ± 0.23	< 0.01*
TAC (um/l)	1.63 ± 0.504	1.9 ± 0.42	< 0.01*
PON1 (um/l)	108.69 ± 39.06	222.8 ± 51.0	< 0.01*

NO nitric oxide, *MDA* malonaldehyde, *TAC* total antioxidant capacity, *PON1* paraoxnase1

*Statistically significant

Table 3 Demonstrating relations between nerve conduction studies and patients' variables

Patients' variables	Group A (n = 38) Number (%)	Group B (n = 22) Number (%)	*p* value
Age ≤ 18 (n = 28)	13 (46.4%)	15 (53.6%)	0.142
Age > 18 (n = 32)	9 (28.1%)	23 (71.9%)	
Male (n = 22)	13 (59%)	9 (41%)	0.603
Female (n = 38)	25 (65.8%)	13 (34.2%)	
On Chelation type (n = 49)	35 (71.4%)	14 (28.6%)	0.013*
No chelation (n = 11)	3 (27.3%)	8 (72.7%)	
Splenectomized (n = 42)	33 (78.6%)	9 (21.4%)	0.002*
Non-Splenectomized (n = 18)	5 (27.8%)	13 (72.2%)	
Serum ferritin < 2000 ng/ml (n = 35)	16 (45.7%)	19 (54.3%)	0.001*
Serum ferritin ≥ 2000 ng/ml (n = 25)	22 (88%)	3 (12%)	

*Statistically significant

underlying direct cause of neuropathy. Our data supported this finding as we found that patients with electrophysiological proof of peripheral motor neuropathy showed elevated serum ferritin, and those with severe iron overload (serum ferritin ≥ 2000 ng/ml) are at higher risk. This was also in line with a previous report of better nerve conduction in well-chelated patients [15]. Our further analyses showed that peripheral neuropathy score (TNSn) correlated positively with transfusion frequency and serum ferritin; highlighting an association between severe iron overload and motor neuropathy which was further proved by the regression analysis model.

B-thalassemia patients were recently proved to have a higher rate of ROS production with subsequent oxidative stress [17, 18]. This may play a role in the pathogenesis of neurological dysfunction [19]. As the nervous system was reported to be especially vulnerable to reactive oxygen species (ROS)-mediated injury due to several reasons including its modest antioxidant defense mechanisms. Free radicals may cause damage of essential macromolecules in the nervous system and culminates in neuronal dysfunction and loss [20]. It is well-known that reactive oxygen species (ROS) are involved in neurodegenerative diseases [21] and several previous studies reported neuroprotective effects of intrinsic and extrinsic antioxidants [22–24]. Our data showed that β-thalassemia patients with abnormal NCS had a disturbed oxidant-antioxidant status in the form of over-consumed TAC and increased oxidative stress. Unfortunately, we failed to prove a direct relationship between oxidant-antioxidant biomarkers and NCS values through the correlation or regression analysis models. In addition, splenectomy was a significant risk factor of motor neuropathy. On the contrary of our results, *Sawaya* et al. [15] reported that splenectomy did not affect the status of the patients' nerves conduction.

In bivariate analysis, age and gender did not appear to be risk or protective factors of neuropathy. However, the amplitude values of Ulnar and Peroneal nerves correlated negatively with age, and peripheral neuropathy score (TNSn) correlated positively with age ($r = 0.4$, $p < 0.01$). This indicates a possible effect of age on the occurrence of neuropathy. Similarly, previous studies reported that neuropathy was worse for older patients [2, 15], irrespective of sex [15].

In conclusion, this study demonstrated a high frequency of a predominantly motor neuropathy in β-thalassemia patients regardless of their age and gender. Severe iron overload may contribute to the pathogenesis of neuropathy.

Other factors including chelation therapy, splenectomy, and oxidative stress might have an enhancing effect that couldn't be proved in the current study.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Statement of Human Rights The current study have been approved by Cairo University research ethics committee (IRB) and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Ethical Approval All procedures performed in the current study were in accordance with the ethical standards of our institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Informed Consent Informed consent was obtained from all individual participants and/or the legal guardians of participants included in the study.

References

1. Weatherall DJ, Clegg JB (1981) The thalassemia syndromes, 3rd edn. Blackwell, Oxford
2. Stamboulis E, Vlachou N, Drossou-Servou M, Tsaftaridis P, Koutsis G, Katsaros N, Loutradi-Anagnostou A (2004) Axonal sensorimotor neuropathy in patients with beta-thalassemia. J Neurol Neurosurg Psychiatry 75:1483–1486
3. Economou M, Zafeiriou DI, Kontopoulos E, Gompakis N, Koussi A, Perifanis V, Athanassiou-Metaxa M (2006) Neurophysiologic and intellectual evaluation of beta-thalassemia patients. Brain Dev 28:14–18
4. Zafeiriou DI, Economy M, Athanasiou-Metaxa M (2006) Neurological complications in beta-thalassemia. Brain Dev 28:477–481
5. Jomova K, Valko M (2011) Importance of iron chelation in free radical-induced oxidative stress and human disease. Curr Pharm Des 17(31):3460–3473
6. Pu YM, Wang Q, Qian ZM (1999) Effect of iron and lipid peroxidation on the development of cerebellar granule cells in vitro. Neuroscience 89(3):855–861
7. Zhao S, Zhang L, Xu Z, Chen W (2013) Neurotoxic effects of iron overload under high glucose concentration. Neural Regen Res 8(36):3423–3433
8. Albers JW, Donofrio PD, McGonagle TK (2016) Nerve conduction manual. e-book; Section 4. Table 1 supplement.Pdf retrieved and downloaded from https://wiki.umms.med.umich.edu/ on Aug 2016
9. Nair V, Turner GA (1984) The thiobarbituric acid test for lipid peroxidation: structure of the adduct with malondialdehyde. Lipids 19:804–805
10. dos Santos AA, Naime AA, de Oliveira J, Colle D, dos Santos DB, Hort MA, Farina M (2016) Long-term and low-dose malathion exposure causes cognitive impairment in adult mice: evidence of hippocampal mitochondrial dysfunction, astrogliosis and apoptotic events. Arch Toxicol 90(3):647–660
11. Dawson B, Trapp RG (2001) Statistical methods for multiple variables. Basic and clinical biostatistics, 4th edn. LANGE Basic Science
12. Logothetis J, Constantoulakis M, Economidou J, Stefanis C, Hakas P, Augoustaki O, Bilek M (1972) Thalassemia major (homozygous beta-thalassaemia). A survy of 138 cases with emphasis on neurologic and muscular aspects. Neurology 22:294–304
13. Zafeiriou DI, Kousi AA, Tsantali CT, Kontopoulos EE, Augoustidou-Savvopoulou PA, Tsoubaris PD, Athanasiou MA (1998) Neurophysiological evaluation of long-term desferrioxamine therapy in beta-thalassemia patients. Pediatr Neurol 18:420–424
14. Papanastasiou DA, Papanicolaou D, Magiakou AM, Beratis NG, Tzebelikos E, Papapetropoulos T (1991) Peripheral neuropathy in patients with beta-thalassaemia. J Neurol Neurosurg Psychiatry 54:997–1000
15. Sawaya RA, Zahed L, Taher A (2006) Peripheral neuropathy in thalassaemia. Ann Saudi Med 26(5):358–363
16. Levine JE, Cohen A, MacQueen M et al (1997) Sensorimotor neurotoxicity associated with high-dose deferoxamine treatment. J Pediatr Hematol Oncol 19:139–141
17. Kattamis C, Lazaropoulou C, Delaporta P, Apostolakou F, Kattamis A, Papassotiriou I (2011) Disturbances of biomarkers of iron and oxidant–antioxidant homeostasis in patients with beta-thalassemia intermedia. Pediatr Endocrinol Rev 8(Suppl 2):256–262
18. Zohaib M, Ansari SH, Hashim Z, Shamsi TS, Zarina S (2016) Serum paraoxonase activity and malondialdehyde serum concentrations remain unaffected in response to hydroxyurea therapy in β-thalassemia patients. J Clin Pharma 56(7):869–874
19. Naithani R, Chandra J, Bhattacharjee J, Verma P, Narayan S (2006) Peroxidative stress and antioxidant enzymes in children with beta-thalassemia major. Pediatr Blood Cancer 46(7):780–785
20. Friedman J (2011) "Why is the nervous system vulnerable to oxidative stress?" Oxidative stress and free radical damage in neurology. Humana Press, New York, pp 19–27
21. Kehrer JP (1993) Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 23:21–48
22. Romay CH, Gonzalez R, Ledon N, Remirez D, Rimbau V (2003) phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. Curr Protein Pept Sci 4(3):207–216
23. Dajas F, Rivera-Megret F, Blasina F, Arredondo F, Abin-Carriquiry JA, Costa G, Morquio A (2003) Neuroprotection by flavonoids. Braz J Med Biol Res 36(12):1613–1620
24. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA (2005) Uric acid and oxidative stress. Curr Pharm Des 11(32):4145–4151