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Original Article

Fumonosin and Sphinganine/Sphingosine Ratio Among Other Parameters as Potential Pathogenic Risk Factors of Neurologic Deficits in Egyptian Children and Adult Renal Patients

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ABSTRACT:

Background: Various pathogenic factors were blamed for the neurologic deficits in renal patients. Fumonosin (FB1) is an occasional mycotoxin that was shown to be responsible for neurologic disturbances in horses by disturbing the sphingolipid metabolism. It was detected as a contaminant among several foodstuffs in Egypt, but was not studied as a risk factor in humans.

Objectives: To study the cross-sectional associations between serum FB1, sphinganine and sphingosine and the prevalence of neurologic deficits among other related factors including serum aluminium (Al), serum parathormone (PTH) levels and other hematological and chemical parameters in a group of children and adult renal patients.

Methods: Visual evoked potential (VEP) was tested in 40 end stage renal disease patients (20 children and 20 adults) on regular dialysis treatment (RDT), 40 patients with different glomerulonephritis (GN) (20 children and 20 adults) and 10 healthy controls. They were studied for serum FB1, sphinganine, sphingosine, calcium, phosphorus, PTH levels, Al, albumin, blood urea, serum creatinine, blood gases and hemoglobin percentage.

Results: FB1 with elevation of sphinganine/sphingosine ratio was found in 12/40 of ESRD patients and 12/40 GN patients but in none of controls ($p < 0.05$). Of the GN group, 9/40 had delayed VEP and 3 of these 9 had positive FB1, but showed no significant correlation with neither FB1 nor PTH ($p > 0.05$). Of the ESRD group, 13/40 had delayed VEP. This delay was detected in 8/12 cases with positive FB1 and in 10/19 cases with more than double the high normal serum PTH and in 13/34 cases with Al more than 20 ug/dl. Al was a sole risk factor in 2/13 ESRD patients with delayed VEP, but neither high PTH nor positive FB1 was encountered solely in any of these cases. In adults ESRD group, VEP abnormalities showed significant correlation with PTH ($p < 0.05$) but not with Al or FB1. Yet, in children it was significantly correlated with the serum PTH, Al and FB1 ($p < 0.004$, 0.03, 0.0001 respectively).

Conclusions: We conclude that long duration of hyperparathyroidism is a risk factor causing neurologic deficits in ESRD patients and may be aggravated by high serum Al and /or a positive FB1, particularly in children.

INTRODUCTION

Chronic renal insufficiency (CRF) is associated with neurological derangement that involves both the central and peripheral nervous systems[(1)]. The clinical features of uremic encephalopathy are non-specific and include confusion, psychomotor agitation, alteration of sleep-wake cycle, disorientation, impaired memory, inattention, paranoid ideation, impaired abstraction, visual hallucination, myoclonus and seizures[(2)]. Patients with CRF on hemodialysis (HD) showed impaired performance on tests of short-term memory, attention, and concentration span and sequential information processing[(3)]. This cognitive decline may or

may not improve with therapy and is associated with diffuse cerebral atrophy, which may be seen on routine studies such as CT and MRI[4].

The uremic toxins responsible for encephalopathy are not exactly defined. Most likely, uremic encephalopathy is a result of combinations of factors, including accumulation of various organic acids and substances such as myoinositol, purines, organic phosphates, oxalates, ascorbic acid, amino acids peptides, parathyroid hormone (PTH), β_2-microglobulin, methylguanidine, guanidosuccinic acid, hippuric acid, polyamines, phenols and indoles, in addition to urea and creatinine[5,6]. Regardless of the level of serum calcium or phosphorus, PTH has been implicated the pathogenesis of uremic encephalopathy because of its effect on neuronal function[7].

Even in the absence of renal insufficiency, elevated levels of PTH can result in confusion and altered mental status[7,8]. The occurrence of dialysis dementia, with impaired cerebrovascular autoregulation and break down of blood-brain barrier, has been linked to aluminium intoxication. CRF patients on regular HD treatment have high serum aluminium levels and may also show increased brain levels especially in the gray mater[4,9]. Aluminium (Al) is normally absorbed from the gastrointestinal tract and is excreted through the kidneys. Increased absorption is probably the effect of PTH on the gastrointestinal tract[10]. Patients on dialysis would have plasma levels that are 6 to 8 times higher than normal (0-20 µg/liter) if exposed to oral Al intake[11].

Anemia is one of the risk factors that cause some neurological deficits. The widespread use of recombinant human erythropoietin (EPO) has led to the realization that some cerebral disturbances of dialysis patients are attributable to anemia. Event related potentials and neuropsychological test scores both improve after treatment with EPO[8]. Finally, changes in brain pH may also play a role in the neurologic dysfunction appearing after dialysis therapy. The syndrome is limited to restlessness, headache, nausea, vomiting, confusion and major seizures[12]. This increase in the intracerebral H^+ ion concentration in the brain results in increased osmolar content with secondary increase in brain water[2].

FB1 is a mycotoxin produced by Fusarium monilliform, a common fungal contaminant of corn throughout the world [13]. It was considered the causative agent of several field out-breaks of equine leucoencephalomalcia (ELEM), with its pathognomonic white matter areas of liquifactive necrosis[14]. FB1 structure resembles sphingoid bases and it inhibits ceramide synthase, altering sphingolipid metabolism [15]. Sphingolipids are important structural components of cells and participate in a variety of processes, including signal modulation[16], oncogenic transformation[17], inter-cellular communication, cell growth, differentiation, proliferation and death[18]. Its human neurologic toxicity has not been studied yet. Some human populations expected to have significant exposure to fumonosin have a high incidence of babies born with neural tube defects[19]. FB1 has been encountered in several foodstuffs in Egypt including yellow corn, cornmeal, white corn, and popcorn by 80%, 53.8%, 33.3%, 27.6% respectively. The level of

FB1 ranged from 10 to 780 μg/kg[20].

The conduction in the tracts of the CNS is vulnerable to toxic factors leading to demyelination of nerve fibers. Visual evoked potential (VEP) was used as an early measure for neurologic deficit in these patients. VEP records provide a way of establishing the presence of demyelinating lesions in central nervous system (CNS). Abnormal responses to pattern stimulation without any eye symptoms indicate that VEP can reveal clinically silent pathology in the visual system and subclinical cases. A complete conduction block may result from extensive demyelinating lesions of the central nervous system. The delay in latency of VEPs may reflect a reduced conduction velocity in damaged visual nerve fibers although some of the prolongation in latency may also be contributed to delay at the cortical or retinal levels. By exclusion, delay in latency in VEP conduction means demyelination in CNS in patients without any ocular lesion. Halliday et al.[21] concluded that VEP amplitude changes presumably reflect a complete conduction block in damaged fibers. Myelination process continues after birth till the age of adolescence and early adulthood[22]. Accordingly, children may be affected more than adults as myelination of the nerve fibers are not completed yet.

AIM OF THE WORK

In this study, we examined the occurrence of FB1 in Egyptian CRF patients on hemodialysis therapy (RDT) and glomerulonephritis (GN) patients with normal or impaired kidney functions and its effect on sphingolipid metabolism and VEP to confirm its role as a possible neurotoxin in renal patients. We also looked at other potential neurotoxic factors in CRF patients including Al intoxication, hyperparathyroidism, anemia and hypoxia.

PATIENTS AND METHODS

In addition to 10 healthy age and sex matched controls, the study included 80 renal patients selected from the adult and pediatric nephrology units at Cairo University Hospitals. They were divided into the following groups:

Group 1 included 20 adults with renal failure on regular dialysis therapy. They were 6 males and 14 females; their mean age was 42.4 ± 11.17 years.

Group 2 included 20 children with renal failure on regular dialysis therapy. They were 12 males and 8 females; their mean age was 11.6 ± 3.15 years.

Group 3 included 20 adult patients (12 males and 8 females with mean age of 36.3 ± 7.8 years) with GN with normal or impaired kidney function on conservative treatment.

Group 4 included 20 children with GN with normal or impaired kidney function on conservative treatment. They were 9 males and 11 females; their mean age was 12.3 ± 2.4 years.

Group 5 included 10 healthy control subjects. They were 4 males and 6 females; their mean age was 27.4 ± 12.6 years, and ranged between 12 and 48 years.

All patients were subjected to full history taking and thorough clinical examination with special emphasis on residence, original disease, presence of hypertension, and symptoms and signs of

neurologic deficits. RDT patients were assessed for the type, frequency, complications occurring during dialysis, and their need for blood transfusion. The patients on conservative treatment were also assessed for any specific treatment received according to their pathologic diagnosis.

Laboratory evaluation

All cases were subjected to the following investigations:

- Kidney function tests (blood urea and serum creatinine), liver function (ALT and AST), serum albumin and total proteins, CBC, serum calcium and phosphorus and serum parathormone level (intact molecule) by enzyme amplified sensitivity immunoassay (EASIA) performed on microtitre plate[23].
- Serum Al level in patients on RDT (group 1, 2) using the direct nitrous oxide-acetylene flame method[24].
- Serum FB1 by thin layer chromatography using C18 plates (5 x 20 cm x 0.25 m) and confirmation was carried out according to Rosttunghaus et al., 1992[25] and serum sphinganine and sphingosine levels by high performance liquid chromatography (HPLC) analysis using an Aminco Bowman Spectrophotoflurometer[26].

Neurophysiologic evaluation

Visual evoked potential (VEP) was measured in all cases as an indicator for the presence of neurologic deficits.

Statistical analysis

All data were expressed as mean ± SD. The statistical analysis of data was performed by analysis of variants (ANOVA) test when data were normally distributed. When the mean values were skewed, Kruskal Wallis test was performed. Pearson coefficient correlation and partial correlation tests were performed to detect the linear relationship between variables. In addition, multiple linear regression analysis was performed to determine the contribution of certain indeper dent parameters to the dependent one (VEP as dependent parameter, PTH, Al, FB1 as independent ones). p values < 0.05 were considered significant. Statistical analysis was performed using SPSS software.

RESULTS

Group1 (adult patients with ESRD on RDT)

The VEP velocity values were significantly delayed than the control group (129.1 ± 33.4 msec, $p < 0.05$) (Table 2). Seven patients had delayed VEP; three of them had high FB1. In addition, PTH levels were significantly elevated among this group (300.8 ± 379.8 ng/dl, $p < 0.05$), ten patients showed high AL levels (36.1 ± 16 μg/dl) and six patients with high FB1 levels (1.34 ± 2.25 ng/μl) (Table 3). There was significant positive correlation between VEP and PTH ($p < 0.05$) (Fig. 1).

Group 2 (children with ESRD on RDT)

In this group, the VEP velocity values were not significantly delayed compared to the control group (122.2 ± 31.9 msec, $p > 0.05$) (Table 2). Six patients had delayed VEP (126.5 ± 37.6 msec); four of them had high FB1 levels. PTH levels were significantly elevated (266.4 ± 262 ng/dl, $p < 0.05$), eleven patients had high Al levels (25.9 ± 16.5 μg/dl) and six had high levels of FB1 (0.93 ± 1.5 ng/ul) (Table 1 and 3). In this group, VEP showed significant positive

correlation with PTH levels ($p < 0.05$) (Fig. 2), Al levels ($p < 0.05$) (Fig. 3) and with FB1 levels which was highly significant ($p = 0.001$) (Fig. 4).

Group 3 (adult patients with GN)

The VEP values were significantly increased when compared with the control response (133.4 ± 26.2 msec, $p < 0.05$) (Table 2). In this group, five patients had delayed VEP; one of them had high level of FB1. PTH level were significantly increased (68.58 ± 68.82 ng/dl, $p < 0.05$) as well as FB1 levels when compared with the control (1.56 ± 2.89 ng/μl, $p < 0.05$) (Table 3). However, there was no significant correlation between VEP and either PTH or FB1 ($p > 0.05$).

Group 4 (Children with GN)

The VEP values were also increased in this group (121.1 ± 28.8) but was not significant compared with the control values ($p > 0.05$) (Table 2). Four patients had delayed VEP; one of those had high FB1. In addition, PTH levels were significantly increased (252.3 ± 290.7 ng/dl, $p < 0.05$) (Table 1). FB1 levels were not significantly increased in this group (0.32 ± 0.9 ng/ul, $p > 0.05$) and there was no significant correlation between VEP and PTH or FB1 ($p > 0.05$).

In addition, FB1 with statistically significant elevation of sphinganine/sphingosine ratio was encountered in 12 from 40 ESRD patients and 12 from 40 GN patients and none of the control ($p < 0.0001$) (Table 3). There was no correlation in all groups between VEP and BUN, creatinine, HB%, PH.

Table 1: Statistical comparison of the biochemical data among studied groups

	Adult CRF on RDT (no. 20)	Children CRF on RDT (no.	Adult GN no. 20	Children GN no. 20	Control no. 10
BUN	56.8 ± 14.2*	59.3 ± 21.1*	35.3 ± 23.9*	35.5 ± 13.2*	11.4 ± 3.4
S.Cr	9.6 ± 3.1*	6.8 ± 2.1*	2.7 ± 2.1*	2.6 ± 1.2*	0.7 ± 0.2
Alb	3.06 ± 0.5*	3.16 ± 0.4*	2.2 ± 0.7*	2.5 ± 0.8*	3.8 ± 0.3
HB%	7.6 ± 1.4*	6.2 ± 1.1*	9.8 ± 2.5*	9.1 ± 1.6*	13.7 ± 1.1
Ca	8.5 ± 1.4	8.7 ± 0.9	8.6 ± 0.8	7.4 ± 1.1*	9.0 ± 0.7
Phos	4.4 ± 1.7*	5.1 ± 1.5*	5.1 ± 1.9*	7 ± 1.8*	3.7 ± 0.4
PTH	300.8 ± 379.8*	266.4 ± 262.5*	68.6 ± 68.8*	252.3 ± 290.7*	41.8 ± 25.8*
Al	36.1 ± 16.4	35.9 ± 16.2			

* $p < 0.005$

Table 2: Visual evoked potential among the studied groups

	Adult CRF on RDT no. 20	Children CRF on RDT no. 20	Adult GN no. 20	Children GN no. 20	Control no. 10
VEP	129.1 ± 33.4*	122.2 ± 31.9	133.4 ± 26.3*	121.1 ± 28.8	102.8 ± 5.1

* $p < 0.05$

Table 3: Serum Fumonisin level and sphinganine/sphingosine ratio among the studied groups

	Adult CRF on RDT no. 20	Children CRF on RDT no. 20	Adult GN no. 20	Children GN no. 20	Control no. 10
FB1	1.34 ± 2.2	0.93 ± 1.5	1.56 ± 2.9*	0.3 ± 0.9	0 ± 0
Sphinganine	46.4*	40.7*	50.8*	29.9*	73.8
Sphingosine	58.9	36.6	63.1*	41.9	8.3
Ratio	38.3*	51.83*	33.6*	38.5*	72.8

* $p < 0.05$

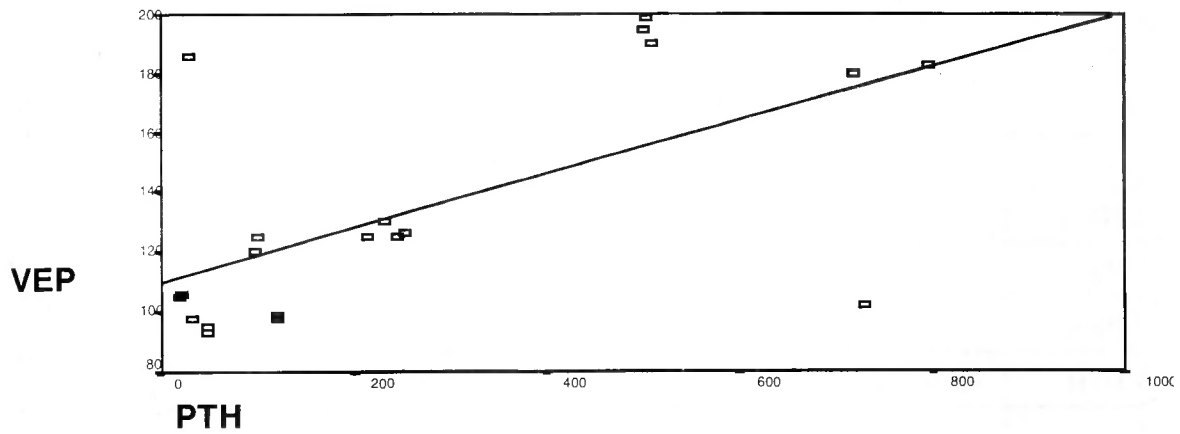

Fig. 1: Correlation between VEP # FB1 in children with CRF on RDT

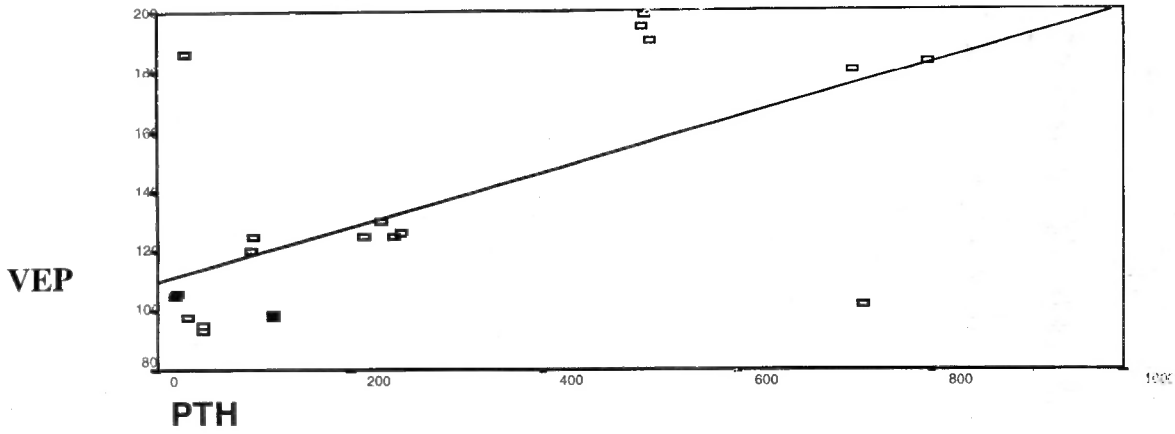

Fig. 2: Correlation between VEP # PTH in children with CRF on RDT

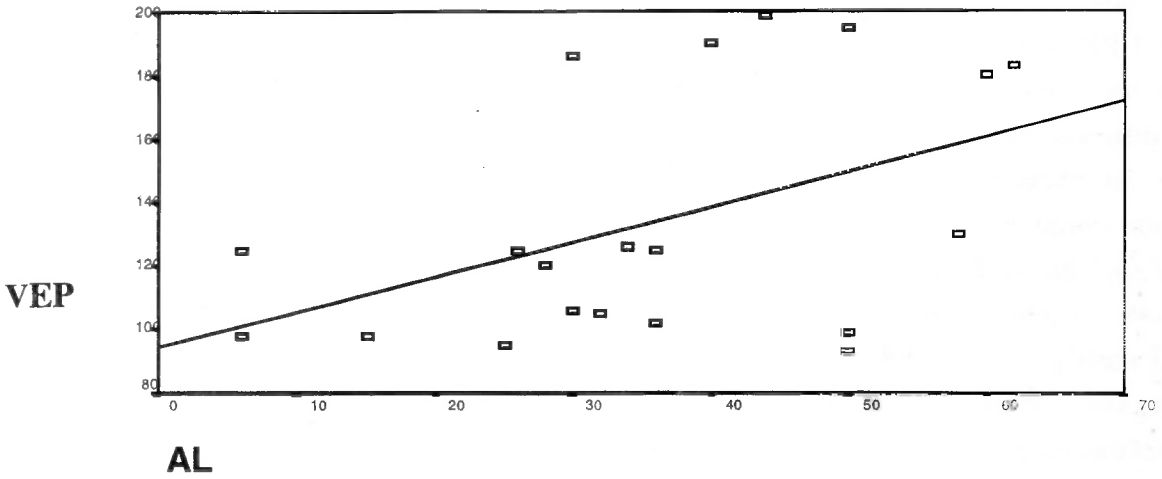

Fig. 3: Correlation between VEP # Al in children with CRF on RDT

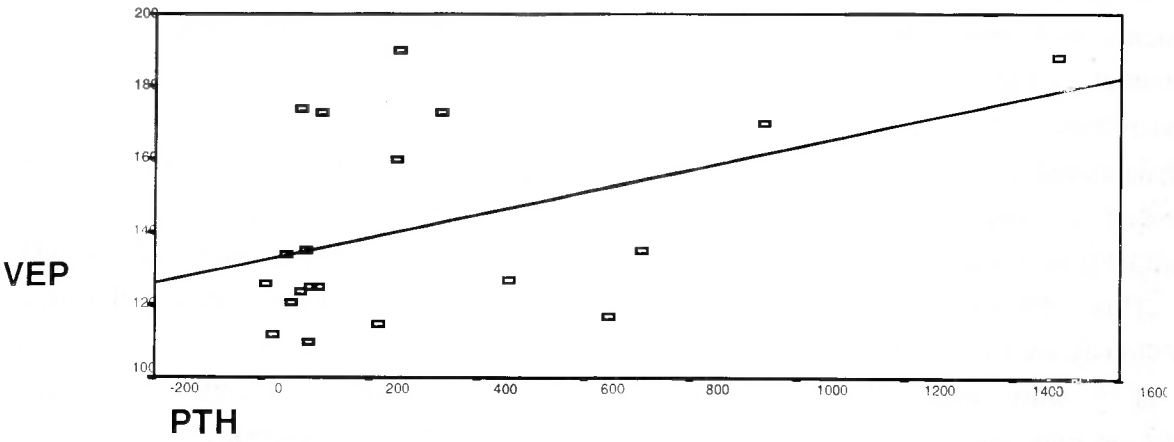

Fig. 4: Correlation between VEP Versus PTH adult patients with CRF on RDT

DISCUSSION

In the present study, we have attempted to define the role of FB1 and its contribution to the neurologic disturbances reported in renal patients among other well described risk factors including uremia, hyperparathyroidism and Al intoxication [5,6,7,9]. VEP measurements in adults whether having CRF on RDT or GN were significantly higher than the controls in our work. This delay in VEP in adult renal patients may be thus reflecting the neurologic pathologic changes induced by various toxic factors that were described previously[4,8,10]. The VEP values in adults with CRF on RDT or GN were also higher than those reported in children. However, the difference was insignificant ($p > 0.05$). This difference could be explained by the longer duration of illness in adults with CRF and the more severe vascuilitic forms of GN in adults compared with children who mostly have minimal change disease. However, there was no significant correlation between the VEP values and the levels of BUN and serum creatinine in both groups. The latter suggests that uremia per se does not play a major role in the neurological deficits observed in renal patients and points to other factors as potential culprits including hyperparathyroidism that could be further substantiated as VEP of adults on RDT showed a significant positive correlation with PTH in this study.

This PTH effect was reported previously by Cogan et al.[8] and Mahoney et al.[12] who suggested a possible link between hyperparathyroidism and neuronal dysfunction. The excess PTH was found to mediate its deleterious effect by causing an elevation in cytosolic calcium of brain cells [27]. In addition, children with CRF on RDT showed a significant positive correlation with PTH suggesting that it could adversely affect the neuronal function through a demyelination process as it showed a greater effect on VEP in children with CRF ($p < 0.005$) in whom the myelination is still progressing than in CRF adults ($p < 0.05$). Furthermore, the lack of significant correlation between the VEP and the PTH levels in patients with GN indicates the importance of the duration of illness as an important pathogenic factor. PTH was reported to alter the level of intracellular calcium that could further affect the myelination and require a longer duration to manifest[12]. In addition, it was the mere presence of the elevated PTH rather than its absolute level that correlated with the delay in VEP, as among the cases with this delay, VEP had no significant correlation with the high PTH level ($p > 0.05$).

In this study, there was no significant correlation between VEP and serum Al levels in adults with CRF on RDT ($p > 0.05$). This finding contrasted with that reported before by Reushe and colleagues[9] who reported a significant link between Al intoxication in CRF patients on RDT and dialysis dementia. However, the lower Al levels and the shorter duration of exposure could explain this different observation. Yet, the VEP in children with CRF on RDT showed a significant correlation with serum Al that could be explained by the fact that the nervous system in children is under development as myelination process is completed by the age of 20[21].

FB1 was reported to contaminate food staff in Egypt[20]. Yet, environmental exposure to FB1 seems insufficient by itself to result in its elevation in the serum unless there is impairment in renal function reducing its excretion as relatives of the patients exposed to the same environmental and nutritional factors were found to have no detectable serum level. FB1 is known to alter the metabolism of sphingolipids, which are important cell membrane structural lipoproteins essential for cellular functions including signal modulation[16]. In this study VEP in children with CRF on RDT showed a significant positive correlation with FB1 levels. The lack of concomitant significant correlation between the VEP in adults with CRF on RDT and FB1 suggested that neurologic conduction in children is more vulnerable to risk factors that are not equally effective in adults. A long exposure to FB1 was significantly positively correlated with VEP in patients with CRF on RDT with a long duration of renal impairment ($p < 0.05$) but had no correlation with it in the GN patients with short duration of illness with or without renal impairment ($p > 0.05$).

The occurrence of FB1 in our cases altered spingolipid metabolism, as it significantly correlated with sphingosine levels in CRF patients on RDT but not in the GN groups. In addition, significant alteration of the sphinganine/sphingosine ratio was observed to occur with elevation of FB1 in both CRF and GN groups suggesting an active pathologic effect of FB1 on the nervous system rather than a mere retention by-stander phenomenon.

In contrast to the described effects of anemia and high intracellular H^+ concentration in the brain cells in inducing neurologic deficits[2], we did not encounter a correlation between VEP and either HB% or pH. However, these factors might affect the metabolic and functional performance of the CNS rather than inducing structural neurologic damage, which would delay the rate of conduction. Furthermore, to exclude the responsibility of nutritional defects as a possible risk factor delaying the VEP in CRF patients, we examined the correlation between VEP and serum albumin, that is known to reflect the nutritional status in RDT patients, which was not statistically significant.

To conclude, this study has shown that hyperparathyroidism is the most important risk factor contributing to the delay of the VEP among patients with CRF on RDT. It also showed that children are more vulnerable than adults to the effects of Al or FB1 intoxication that requires more meticulous care concerning water treatment, more frequent analysis of serum Al and avoiding any suspicious contaminants whenever possible.

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