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ORIGINAL PAPER

Association of Serum Trace Elements and Minerals with Genetic Generalized Epilepsy and Idiopathic Intractable Epilepsy

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Abstract Certain minerals and trace elements are essential for the development of healthy nervous system. Altered serum levels of these elements may lead to the development of various diseases including epilepsy. The present study was designed to evaluate the association of serum calcium, magnesium, zinc and copper in the development of genetic generalized epilepsy [GGE; erstwhile known as idiopathic generalized epilepsy (IGE)] as well as idiopathic intractable epilepsy (IIE), in which seizures persist despite treatment with at least two or three antiepileptic drugs tolerated at reasonable dosage. 200 GGE patients and equal number of healthy controls were recruited for study with their written informed consent. The patients were further divided into responders and non-responders based on their response to antiepileptic drugs. Copper and zinc levels were assayed by atomic absorption spectrophotometer whereas calcium and magnesium were analyzed by Human Star 600 fully automated biochemistry analyzer. The patients with GGE had significant low levels of calcium, magnesium and zinc (1.85 ± 0.33, 0.69 ± 0.13 mmol/L and 11.33 ± 3.32 μmol/L respectively) and the corresponding values for controls were 2.27 ± 0.22, 0.89 ± 0.15, 12.71 ± 3.24 ($p < 0.05$). Significant high levels of copper were found in patients as compared to controls (26.69 ± 8.79 μmol/L; 16.64 ± 3.64) ($p < 0.05$). Significantly decreased levels of zinc were noted in non-responders (10.38 ± 2.99) compared to responders (12.62 ± 3.30) ($p < 0.05$). No significant difference was observed in serum calcium, magnesium and copper levels between responders and non-responders. In conclusion, low levels of calcium, magnesium, zinc and high levels of copper were found to be associated with GGE. Further, the patients with IIE were also found to have low levels of zinc.

Keywords Genetic generalized epilepsy (GGE) · Idiopathic intractable epilepsy (IIE) · Trace elements · Minerals · Anti-epileptic drugs (AEDs)

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Introduction

Epilepsy is one of the most common chronic neurological disorders manifesting as sudden, recurrent and unprovoked epileptic seizures with a prevalence of 3–6/1,000 world population [1]. Genetic generalized epilepsy (GGE) is a common form of epilepsy, clinically characterized by absence, myoclonic seizures, and tonic–clonic seizures with the electroencephalographic (EEG) pattern of

bilateral, synchronous, and symmetrical spike and wave or polyspike and wave discharges. Four main GGE subsyndromes namely childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and GGE with tonic–clonic seizures alone (GTCA) have been identified based on predominant seizure type and age of onset [2, 3]. The exact pathogenesis of idiopathic seizure is not fully understood. Several factors such as neurotransmitters, transporters, granule cells, voltage gated ionic currents and non-neural proliferations may play a key role for epilepsy [4, 5]. However, any mutation or defect in mitochondrial respiratory chain complexes, synapses, and neurotransmitter receptors or in the voltage and ligand channels may alter brain excitability and cause epileptic seizures [6].

The ionic regulatory system (IRS) comprises network of pumps, channels and exchangers. When the neuron is at rest, this system runs in a steady state and responds well to increase neuronal signaling by resuming normal ion distributions. The IRS has the ability to recognize intra and extra-cellular ions rapidly, but this can be overwhelmed by excessive activity, allowing ion concentration to drift towards equilibrium permitting their effects to terminate neuronal dynamics [7, 8]. Certain minerals and trace elements are essential for the development of healthy nervous system and neuronal susceptibility to excitability and the movement of trace elements across cell membranes, between extra and intracellular fluid is responsible for the primary functions of body. Altered homeostasis of some trace elements in the brain may be involved in the susceptibility, development and termination of seizures in animal models of genetically determined epilepsy [9].

Previous reports have suggested that altered levels of trace elements and minerals play an important role in the pathophysiology and recurrence of seizures [9–12]. Voltage-gated calcium channels are integral membrane proteins that form calcium selective pores in the plasma membrane. In neurons, the rapid influx of calcium depolarizes the cell membrane potential and mediates action potential, membrane potential oscillations and regulates the intracellular signaling pathways as well as neurotransmitter release. Any alteration in their expression can cause pathophysiological changes in the brain in turn leads to epileptic seizures [13]. Influx of calcium and decreased K^+ and Cl^- transport mechanisms contribute to the increased membrane excitability [14]. Excitotoxicity can be achieved by pathologically increased levels of glutamate, excitotoxins like NMDA and kainic acid by allowing high levels of calcium ions to enter the cell [15]. This influx activates number of enzymes like phospholipases, endonucleases and proteases that may lead to cell damage. The pathological variations in serum calcium levels have been reported to result in epileptic convulsions [16, 17].

Magnesium is an essential element having a role in neuronal excitability. It helps in slowing the electric discharge as well as its spread in the brain. Hence depletion of magnesium can lead to hyper excitability of neurons [18, 19]. Low Mg can reduce surface charge of neuronal membrane, thereby increasing neuronal hyper excitability [20].

Zinc is abundant in brain especially in hippocampus and an important element for normal neuronal communication as well as proper functioning of the inhibitory neurotransmitter, gamma amino butyric acid (GABA). Zinc may play a role in pyridoxal phosphate-mediated regulation of glutamic acid decarboxylase, the key enzyme in the synthesis of GABA [17, 21]. Altered zinc metabolism has been found to play a role in the pathology of epilepsy. Low levels of serum zinc may play an important role in the induction epileptic seizures via activating NMDA receptors [10, 22].

Copper is an important trace element that acts as a co-factor for different enzymes. Copper in low dosage has been reported to produce seizures in animals via inhibiting Mg ATPase and Na, K ATPase enzymes. Copper inhibits Mg^{2+} adenosine triphosphatase and Na^+ K^+ATPase enzymes thereby disturb Na K homeostasis. This further leads to epileptiform discharges [23, 24].

Some studies have been carried out to evaluate the association of various minerals and trace elements with febrile seizures [25–27]. However, there are very few reports on the association of these minerals and trace elements with GGE. Therefore, the current study was designed to evaluate the association of serum levels of minerals such as calcium and trace elements like magnesium, zinc and copper with GGE as well as IIE in a South Indian population from Andhra Pradesh.

Methods

Two hundred diagnosed epileptic patients of GGE (male:female = 131:69) along with age and sex matched healthy controls were recruited for this study with their written informed consent. The study was approved by the Ethical Committee of the 'Institute of Genetics and Hospital for Genetic Diseases', as well as from the study hospital. The clinical diagnosis was made according to the admitted criteria by International League Against Epilepsy by an expert epileptologist from tertiary care centre on the basis of age of onset, predominant type of seizures being generalised seizures in addition to myoclonic seizures, absence seizures and on EEG findings. In addition patient should have normal intelligence and the MRI Brain has to be normal. The seizures usually are infrequent. Classical EEG findings are normal background with generalised

spike and wave discharges typical of individual syndrome type and having photosensitivity and activation during hyperventilation. For childhood absence/juvenile absence epilepsy, patients had typical absences along with classical generalised spike and wave or polyspike and wave discharges of 3 Hz (>2.5 to <4.5 Hz) For juvenile myoclonic epilepsy, patient had myoclonic jerks with poly spike and wave discharges of >4.5 Hz frequency. For GGE, the discharges are 3–4.5 Hz frequency. There can be positive family history. The information on demographic features such as socioeconomic status, nutritional status and geographic area were collected in a specially designed proforma. The seizure types, medical history were also obtained about each patient. The subjects were on their normal dietary regime. The patients were further divided into two sub-groups, responders and non-responders on the basis of their response to prescribed antiepileptic drugs (AEDs). Non-responders included the patients who had one or more seizures in the previous 6 months despite being treated with at least two antiepileptic drugs such as phenytoin, carbamazepine, sodium Valproate etc.

Three milliliters of venous blood was collected from all the subjects in sterile silicone tubes without addition of anticoagulants and centrifuged at 2,500 rpm for 20 min. The serum was collected in sterile tubes. The tubes were stored at −80 °C until the levels of serum Ca, Mg, Zn and Cu were analysed. Estimation of copper and zinc is done by AA-700 atomic absorption spectrophotometer with auto sampler (Shimadzu, Japan) using an acetylene flame and hollow cathode lamps. Sera were diluted with distilled water (1:4). The analysis wavelengths were 324.8 and 213.9 respectively [28, 29] whereas calcium and magnesium were estimated by Human Star 600 fully automated biochemistry analyser using Human kits, Germany. The difference in the levels of minerals and trace elements was evaluated by Student's *t* test and the *p* value of <0.05 was considered to be significant.

Results

Two hundred patients with GGE and 200 age and sex matched healthy controls were included in this study. The mean ± SD age (in years) of the patients with GGE was 24.9 ± 15.1 and the healthy controls were 22.9 ± 16.8 (Table 1). The family history of GGE was observed in 23 % patients (Table 1). Out of 200 GGE patients, in majority the age of onset was <15 years (110 patients 55 %) and remaining 90 patients (45 %) had the onset above the age of 20 years. The mean time since diagnosis was 14.8 years. The type of seizures in different age groups has been given in Table 2. Serum Ca, Mg, Zn and Cu levels in patient and control groups are given in Table 3.

Table 1 Demographic features of GGE patients and controls

Demographic features	Patients (n = 200)	Controls (n = 200)
Age [years (mean ± SD)]		
<15	8.1 ± 2.47	10.5 ± 4.5
>15	38.3 ± 12.2	37.0 ± 10.0
Sex (male:female)	131:69	131:69
Family history (%)	23	2

Table 2 Seizure types in different age groups in GGE patients

Seizure type	Age of onset <15 years (n = 110)	Age of onset >15 years (n = 90)
Absence	40 (36.4 %)	2 (2.2 %)
Myoclonus	8 (7.2 %)	72 (79.9 %)
Tonic–clonic	102 (92.7 %)	88 (97.8 %)

When gender and age were not taken into account in the patient group, the mean ± SD levels of Ca, Mg, Zn and Cu were 1.85 ± 0.33, 0.69 ± 0.13 mmol/L, 11.33 ± 3.32 and 26.69 ± 8.79 μmol/L respectively. The corresponding values for control group were 2.27 ± 0.22, 0.89 ± 0.15, 12.71 ± 3.24 and 16.64 ± 3.64 respectively. The mean ± SD serum levels of Ca in patients were significantly lower (1.85 ± 0.33) when compared to healthy controls (2.27 ± 0.22) ($p < 0.001$). A significant difference was found in the serum Mg levels which were low in patients (0.69 ± 0.13) as compared to controls (0.89 ± 0.15) ($p < 0.001$). Similar trend was observed in case of serum levels of Zn also. Statistically significant lower levels of zinc were observed in patients (11.33 ± 3.32) when compared to healthy controls (12.71 ± 3.24) ($p = 0.001$). However, we found higher levels of Cu in patient group (26.69 ± 8.79) in comparison with the control group (16.64 ± 3.69) ($p < 0.001$). A significant difference was observed in the serum calcium of female patients when compared to males (1.78 ± 0.30, 1.90 ± 0.32) ($p = 0.01$). However, no significant difference was noted in the serum levels of magnesium, zinc and copper between males and females. There was no significant difference in serum levels of Ca, Mg, Zn and Cu between different age groups. All the patients were either on mono-or polytherapy. The drugs prescribed included Valproate, Eptoin, Lamotrigine, Carbamazepine and Levetiracetam. Significant low levels of serum zinc were noticed in patient sub group, non-responders (10.38 ± 2.99) compared with responders (12.62 ± 3.30) ($p < 0.001$). However, no significant difference was observed in serum calcium, magnesium and copper levels between responders and non-responders (1.91 ± 0.31, 1.82 ± 0.32; 0.67 ± 0.12, 0.69 ± 0.13 and 26.53 ± 9.31,

Table 3 Serum calcium, magnesium, zinc and copper levels in GGE patients and controls

Parameter	Patients (n = 200, mean ± SD)	Controls (n = 200, mean ± SD)	*p* value
Calcium (mmol/L)	1.85 ± 0.33	2.27 ± 0.22	<0.001*
Magnesium (mmol/L)	0.69 ± 0.13	0.89 ± 0.15	<0.001*
Zinc (μmol/L)	11.33 ± 3.32	12.71 ± 3.24	<0.001*
Copper (μmol/L)	26.69 ± 8.79	16.64 ± 3.64	<0.001*

* $p < 0.05$ significant

26.69 ± 8.41 and $p = 0.06$, $p = 0.43$ and $p = 0.86$ respectively). The serum levels of various minerals and trace elements in different epilepsy types have been given in Table 4. A significant difference was observed in Zn and Mg levels between the different types of epilepsy.

Discussion

The exact pathogenesis of GGE is not fully understood. However, there are studies suggesting that the body electrolytes, the level of some trace elements and the membrane lipid peroxidation due to increased free radicals or decreased anti-oxidant defense mechanisms have been causally involved in some forms of epilepsies and contribute to pathophysiology of neuronal excitability, neuronal excitotoxicity, seizure recurrence and resistance to AEDs [9, 11]. Seizures are followed by significant alterations in both intra and extra-cellular ion concentrations [30, 31]. With the involvement of metallic elements, the processes like oxidative stress, excitotoxicity, mitochondrial dysfunction or protein aggregation may lead to the atrophy and death of neurons in some neurodegenerative disorders [32–34]. As the epileptic seizures induce neurodegeneration, it is assumed that metals may be involved in the progress of epilepsy [9].

Magnesium is a potential modulator of seizure activity because of its ability to antagonize the excitatory calcium influx through NMDA receptor [35, 36] and it was also demonstrated that low levels of magnesium can increase the frequency of seizures in the patients with refractory epilepsy that is considered to be the most important risk factor for occurrence of sudden unexpected death in epilepsy (SUDEP) [37, 38]. It was also noted that the deficiency of magnesium decreases the seizure thresholds in animal models of epilepsy [39]. In addition to this, magnesium sulphate is an effective therapeutic agent commonly used to prevent and to arrest the recurrence of seizures of eclampsia and pre-eclampsia [40, 41]. More recently, case reports have also been described seizures due to hypomagnesaemia in infants and adults [42, 43]. In the present study, significant low levels of serum magnesium were observed in GGE patients compared to the age and sex matched healthy controls ($p < 0.0001$). Our results are in agreement with a previous study carried out by Sood et al., who reported a significant reduction in serum magnesium levels in patients with GGE when compared to age and sex matched controls [44]. It has been reported that the deficiency/reduced levels of magnesium might lead to the seizure susceptibility or epilepsy [45, 46] because the reduced Mg concentration can hyper excite the neurons [18, 47]. In another study by Oladipo et al., the plasma Mg concentrations were significantly lower in those with epilepsy than in controls [48].

Zinc is an essential trace element that participates in the structure of superoxide dismutase and glutathione peroxidase enzymes which are known to be involved in antioxidative defense mechanisms. Normally, the free zinc is concentrated in pre-synaptic vesicles of glutamatergic neurons [49, 50] and colocalized to GABA and glycine containing murine neurons [51]. In pathological conditions, the free zinc that is in excess will be released from synaptic pools where it exploits α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors to get into the neurons [52–54]. Under normal physiological conditions zinc and other trace elements have been found

Table 4 Serum calcium, magnesium, zinc and copper levels in different epilepsy types

Parameter	Absence epilepsy (CAE + JAE) (n = 58)	Myoclonic epilepsy (JME) (n = 92)	TC seizures (GGE + GTCS on awakening) (n = 50)	*p* value
Calcium (mmol/L)	1.80 ± 0.31	1.85 ± 0.35	1.92 ± 0.32	0.28
Magnesium (mmol/L)	0.63 ± 0.12	0.72 ± 0.13	0.69 ± 0.15	<0.001*
Zinc (μmol/L)	11.9 ± 3.77	9.4 ± 2.18	12.6 ± 4.0	<0.001*
Copper (μmol/L)	26.53 ± 8.69	27.0 ± 8.52	26.53 ± 9.13	0.93

* $p < 0.05$ significant

to modulate the neuronal excitability. Enhanced seizure susceptibility has been reported in Zn deficient mice whereas it lowers in Zn supplemented mice. No change has been observed in mice with adequate dietary zinc [55]. In the present study, significant low levels of serum zinc were observed in GGE patients compared to healthy controls ($p < 0.001$). Our results are in accordance with previous studies where significant lower levels of serum zinc have been reported in epileptics [11, 56, 57]. Hypozincemia may facilitate the seizure activity by inhibiting the inhibitory neurotransmitter, GABA or by defective production of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Low levels of serum zinc have also been reported in children with febrile seizures [25, 58, 59]. Some studies have also reported slightly higher levels of serum zinc in GGE cases compared to controls [17, 60]. Role of zinc in seizure is controversial as it plays a role in the synthesis and function of inhibitory neurotransmitter, GABA at one hand; it also has an inhibitory effect on GABA on the other hand and thus facilitates seizure activity [61]. Excess zinc has been implicated in the production of free radicals as well as reactive oxygen species (ROS) which can cause neuronal damage and death [62]. In addition, the hypozincemia activates NMDA receptors which may play an important role in the induction of epileptic discharge. However, Atilla et al., found no significant difference in serum zinc between cases and controls [63].

Copper is an important trace element that acts as a co-factor for different enzymes. It inhibits Mg ATPase and Na, K ATPase enzymes that disturbs Na, K homeostasis which in turn alleviates the development of epileptic seizure [23]. It was also known that at high concentrations copper can produce oxidative damage to biological systems, including peroxidation of lipids or other macromolecules [64] which is a contributing factor for the development of epilepsy [9, 11]. The present study shows significant higher levels of serum copper in GGE patients compared to healthy controls ($p < 0.001$). This is in accordance with previous studies where elevated serum copper levels in GGE patients have been reported [11, 17, 65]. However, Saad et al. (2013) did not find any significant variation in copper levels among epileptic patients and controls [57].

Calcium is an essential element that plays a major role in the normal functioning of neurons and at neuromuscular junction. Due to the depolarization, the excitability of neurons increases the extra cellular glutamate concentration reflecting increased intracellular neuronal calcium which further depolarizes the cell leading to neuronal injury and or death under conditions of excessive neuronal activation [13]. High Ca^{2+} concentration in the epileptic neurons remains elevated during acute injury phase as well as chronic epilepsy phase, and plays a role in maintenance of the spontaneous recurrent seizures. It can alter GABAA receptor recycling which can serve as a possible mechanism for the effect of Ca^{2+} on altering neuronal excitability [66]. In the present study also low levels of serum calcium were observed in GGE patients when compared to control group ($p < 0.001$). In a prospective case control study carried out by Oladipo et al. (2007), calcium levels were found to be lower in the epileptic patients [16]. The mechanism suggested for lower calcium levels or hypocalcaemia leading to the development of seizures is that hypomagnesaemia causes serum calcium levels to decline in a significant way by impairing the synthesis or secretion of parathyroid hormone [67, 68] and together they produce a membrane state of hyper excitability of neurons, which have been found to be associated strongly with seizures in adults and children [16]. Leaver et al. also observed low calcium levels in epileptic patients [69]. It has also been reported that massive accumulation of calcium in brain occurs due to the rapid inflow of calcium ions from blood to tissue [70]. This also may be the possible reason for lower levels of serum calcium in GGE patients. Natelson et al. (1979) assumed that the release of hormones, epinephrine and corticotrophin during stress, contributes to low levels of serum calcium just before a seizure [71].

Most of the epileptic seizures can be controlled by medical therapy with AEDs but 1/3rd of the patients do not respond and so that they suffer from intractable epilepsy [72, 73]. We categorized patients with Genetic Generalized Epilepsy into 2 sub-groups viz. responders and non-responders (who have developed at least one seizure attack in previous 6 months despite being treated with 2 AEDs). We found significant low serum zinc levels in non-responders when compared to responders ($p < 0.001$). Seven et al. [12], also reported low levels of serum zinc in non-responders and claimed the deficiency of serum zinc is a causative factor for the idiopathic intractable epilepsy. However, we did not find any significant difference in the serum levels of calcium, magnesium and copper with patient sub groups, responders and non-responders ($p = 0.06$, $p = 0.43$ and $p = 0.86$) respectively. The risk of drug resistance (intractable epilepsy) was significantly higher in non-responder patient group with low levels of serum zinc.

In conclusion, our results suggest that the low levels of serum calcium, magnesium, zinc and high levels of are associated with GGE. However, further studies are required to examine the association of trace elements in the pathogenesis of GGE. Our results also indicate that zinc deficiency might be associated with idiopathic intractable epilepsy.

References

1. Begley CE, Baker GA, Beghi E, Butler J, Chisholm D, Langfitt JT et al (2007) Cross-country measures for monitoring epilepsy care. Epilepsia 48:990–1001
2. Commission report. Commission on classification and terminology of the international league against epilepsy (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 30:389–399
3. Andermann F, Berkovic SF (2001) Idiopathic generalised epilepsy with generalised and other seizures in adolescence. Epilepsia 42:317–320
4. Berkovic SF (1998) Neurobiology of epilepsy. In: Eugel J Jr, Pedley TA (eds) Epilepsy: a comprehensive textbook. Lippincott-Raven, Philadelphia, pp 217–224
5. Afawi Zaid, Suls Arvid, Ekstein Dana, Kivity Sara, Neufeld Miriam Y, Oliver K (2010) Mild adolescent/adult onset epilepsy and paroxysmal exercise-induced dyskinesia due to GLUT1 deficiency. Epilepsia 51(12):2466–2469
6. Martinc B, Grabnar I, Vovk T (2012) The role of reactive species in epileptogenesis and influence of antiepileptic drug therapy on oxidative stress. Curr Neuropharmacol 10:328–343
7. Ullah G, Cressman JR, Barreto E, Schiff SJ (2009) The influence of sodium and potassium dynamics on excitability, seizures, and the stability of persistent states: II.Network and glial dynamics. J Comput Neurosci 26(2):171–183. doi:10.1007/s10827-008-0130-6
8. Ransom CB, Ransom BR, Sotheimer H (2000) Activity dependent extracellular K^+ accumulation in rat optic nerve: the role of glial and axonal Na^+ pumps. J Physiol 522:427–442
9. Hamed SA, Abdellah MM, El-Melegy N (2004) Blood levels of trace elements, electrolytes, and oxidative stress/antioxidant systems in epileptic patients. J Pharmacol Sci 96:349–359
10. Lee SW, Chung SS (2010) A review of the effects of vitamins and other dietary supplements on seizure activity. Epilepsy Behav 18:139–150
11. Wojciak RW, Mojs E, Stanislawska-Kubiak M, Samborski W (2013) The serum zinc, copper, iron, and chromium concentrations in epileptic children. Epilepsy Res 104(1):40–44
12. Seven M, Basaran Sarenur Y, Mujgan C (2013) Deficiency of selenium and zinc as a causative factor for idiopathic intractable epilepsy. Epilepsy Res 104:35–39
13. Cain SM, Snutch TP (2012) Voltage-gated calcium channels in epilepsy. In: Noebels JL, Avvoli M, Ragawski MA, Olsen RW, Delgado-Escuete AV (eds) Jasper's basic mechanisms of the epilepsies, 4th edn. National Center for Biotechnology Information (US), Bethesda (MD)
14. Kim AH, Kerchner GA, Choi DW (2002) Blocking excitotoxicity or glutamatergic storm. In: Marcoux FW, Choi DW (eds) CNS neuroprotection, chapter 1. Springer, New York, pp 3–36
15. Manev H, Favaron M, Guidotti A, Costa E (1989) Delayed increase of Ca^{2+} influx elicited by glutamate: role in neuronal death. Mol Pharmacol 36(1):106–112
16. Oladipo OO, Lesi FE, Ezeaka VC (2007) Plasma magnesium and calcium levels in children with epilepsy in lagos. NGGEr Postgrad Med J 14(1):26–29
17. Prasad R, Singh A, Das BK, Upadhayay RS, Singh TB, Mishra OP (2009) Cerebrospinal fluid and serum Zn, Cu, Mg and Ca levels in children with idiopathic seizures. J Clin Diagn Res 3(6):1841–1846
18. Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurons. Nature 309:261–263
19. Zhou FW (2014) TRPC3 mediates hyperexcitability and epileptiform activity in immature cortex and experimental cortical dysplasia. J Neurophysiol 111(6):1227–1237
20. Isaev D, Ivanchick G, Khmyz V, Isaeva E, Savrasova A, Krishtal O, Holmes GL, Maximyuk O (2012) Surface charge impact in low-magnesium model of seizure in rat hippocampus. J Neurophysiol 107(1):417–423
21. Ebadi M, Itoh M, Bifano J, Wendt K, Earle A (1981) The role of Zn^{2+} in pyridoxal phosphate mediated regulation of glutamic acid decarboxylase in brain. Int J Biochem 13(10):1107–1112
22. Shah QA, Jamil AA, Gupta VP, Kabiraj MM, Shah AH (2001) Changes in serum electrolytes in childhood epilepsy: a hospital based prospective. Greenwich J Sci Tech 2:18–27
23. Donalson J, St Pierre T, Minnich J, Barbeau A (1971) Seizures in rats associated with divalent cation inhibition of Na+-K+-ATPase. Can J Biochem 49:1217–1224
24. Barbeau A, Donaldson J (1974) Zinc, taurine and epilepsy. Arch Neurol 30:52–58
25. Ganesh R, Lalitha J (2008) Serum Zinc level in children with simple febrile seizure. Clin Pediatrics 47(2):164–166
26. Ahmad T, Zarichehr V, Safar AT, Seyede MK, Gholam AM (2009) Assessment of the relation between serum zinc and magnesium levels in children with febrile convulsion. Iran J Pathol 4(4):157–160
27. Amiri M, Farzin L, Moassesi ME, Sajadi F (2010) Serum trace element levels in febrile convulsion. Biol Trace Elem Res 135:38–44
28. Beaty RD, Kerber JD (1993) Concepts, instrumentation and techniques in atomic absorption spectroscopy, 2nd edn. Perkin Elmer Corporation, Norwalk, pp 1–8
29. Perkin R, Elmer C (1996) Analytical methods for atomic absorption spectroscopy. The Perkin Elmer Corporation, USA, pp 5–6
30. Hotson JR, Sypert GW, Ward AA (1973) Extra cellular potassium concentration changes during propagated seizures in neocortex. Exp Neurol 38:20–26
31. Kager H, Wadman JW, Somjen GG (2000) Simulated seizures and spreading depression in a neuron model incorporating interstitial space and ion concentrations. J Neurophysiol 84(1):495–512
32. Bush AI (2000) Metals and neuroscience. Curr Opin Chem Biol 4:184–191
33. Sayre LM, Perry G, Smith MA (1999) Redox metals and neurodegenerative disease. Curr Opin Chem Biol 3(2):220–225
34. Campbell A, Smith MA, Sayre LM, Bondy SC, Perry G (2001) Mechanisms by which metals promote events connected to neurodegenerative diseases. Brain Res Bull 55:125–132
35. Sinert R, Zehtabchi S, Desai S, Peacock P, Altura BT, Altura BM (2007) Serum ionized magnesium and calcium levels in adult patients with seizures. Scand J Clin Lab Invest 67:317–326
36. Hallak M (1998) Effect of parenteral magnesium sulfate administration on excitatory amino acid receptors in the rat brain. Magnes Res 11:117–131
37. Nashef L, Ryvlin P (2009) Sudden unexpected death in epilepsy (SUDEP): update and reflections. Neurol Clin 27:1063–1074
38. Surges R, Thijs RD, Tan HL, Sander JW (2009) Sudden unexpected death in epilepsy: risk factors and potential pathomechanisms. Nat Rev Neurol 5:492–504
39. Aw Yuen, Sander JW (2012) Can magnesium supplementation reduce seizures in people with epilepsy? Hypothesis. Epilepsy Res 100(1–2):152–156
40. Lu JF, Nightingale CH (2000) Magnesium sulfate in eclampsia and pre-eclampsia:pharmacokinetic principles. Clin Pharmacokinet 38(4):305–314
41. Omu AE, Al-Harmi J, Vedi HL, Mlechkova L, Sayed AF, Al-Ragum NS (2008) Magnesium sulphate therapy in women with pre-eclampsia and eclampsia in Kuwait. Med Princ Pract 17(3):227–232
42. Fagan C, Phelan D (2001) Severe convulsant hypomagnesaemia and short bowel syndrome. Anaesth Intensiv Care 29:281–283

43. Weisleder P, Tobin JA, Kerrigan JF III, Bodensteiner JB (2002) Hypomagnesemic seizures: case report and presumed pathophysiology. J Child Neurol 17:59–61
44. Sood AK, Handa R, Malhotra RC, Gupta BS (1993) CSF, RBC, Urinary levels of magnesium and calcium in idiopathic generalized tonic clonic seizures. Indian J Med Res 98:152–159
45. Rude RK (1993) Magnesium metabolism and deficiency. Endocrinol Metab Clin N Am 22:377–395
46. Malik PA, Politzer N, Carlen PL (2012) Magnesium as an effective adjunct therapy for drug resistant Seizures. Can J Neurol Sci 39(3):323–327
47. Furukawa Y, Kasai N, Torimitsu K (2009) Effect of Mg2+ on neural activity of rat cortical and hippocampal neurons in vitro. Magnes Res 22(3):174S–181S
48. Oladipo OO, Ajala MO, Okubadejo N, Danesi MA, Afonja OA (2003) Plasma magnesium in adult NGGErian patients with epilepsy. NGGEr Postgrad Med J 10:234–237
49. Danscher G (1996) The autometallographic zinc-sulphide method. A new approach involving in vivo creation of nanometer-sized zinc sulphide crystal lattices in zinc-enriched synaptic and secretory vesicles. Histochem J 28(5):361–373
50. Franco-Pons N, Casanovas-Aguilar C, Arroyo S, Rumi'a J, Ṕerez-Clausell J, Danscher G (2000) Zinc-rich synaptic boutons in human temporal cortex biopsies. Neuroscience 98(3):429–435
51. Kovács KJ, Larson AA (1997) Zn2+ inhibition of [3H]MK-801 binding is different in mouse brain and spinal cord: effect of glycine and glutamate. Eur J Pharmacol 324(1):117–123
52. Sensi SL, Canzoniero MT, Yu SP, Ying HS, Koh J et al (1997) Measurement of intracellular free zinc in living cortical neurons: routes of entry. J Neurosci 17(24):9554–9564
53. Weiss JH, Sensi SL (2000) Ca2+ -Zn2+ permeable AMPA or kainite receptors: possible key factors in selective neurodegeneration. Trends Neurosci 23(8):365–371
54. Marťınez-Gaĺan JR, Díaz C, Juiz JM (2003) Histochemical localization of neurons with zinc-permeable AMPA/kainite channels in rat brain slices. Brain Res 963(1–2):156–164
55. Fukahori M, Itoh M (1990) Effects of dietary zinc status on seizure susceptibility and hippocampal zinc content in the El (epilepsy) mouse. Brain Res 529(1–2):16–22
56. Farahani Heidar N, Ashthiani Alireza R, Masihi Mher S (2013) Study on serum zinc and selenium levels in epileptic patients. Neurosciences 18(2):138–142
57. Saad K, Hammad E, Hassan AF, Badry R (2014) Trace element, oxidant and antioxidant enzyme values in blood of children with refractory epilepsy. Int J Neurosci 124(3):181–186
58. Mishra OP, Singhal D, Upadhyay RS, Prasad R, Atri D (2007) Cerebrospinal fluid zinc, magnesium, copper and gamma-aminobutyric acid levels in febrile seizures. J Pediatric Neurol 5:39–44
59. Ehsanipour F, Talebi-Taher Harandi NV (2009) Serum zinc level in febrile convulsion and its complications with that of control group. Iran J Pediatrics 19(1):65–68
60. Kumar Lalit, Chaurasiya OS, Gupta AH (2011) Prospective study of level of serum zinc in patients of febrile seizures, idiopathic epilepsy and CNS infections. People's J Sci Res 4(2):1–4
61. Kumar L, Chaurasiya OS, Gupta AH (2011) Prospective study of level of serum zinc in patients of febrile seizures, idiopathic epilepsy and CNS infections. People's J Sci Res 4:1–4
62. Frazzini V, Rockabrand E, Mocchegiani E, Sensi SL (2006) Oxidative stress and brain aging: is zinc the link? Biogerontology 7(5–6):307–314
63. Atilla II, Elit GO, Mukaddes G, Bunyamin IIK, Nevin II, Neciip YI, Omer A (2004) The comparison of nail and serum trace elements in patientswith epilepsy and healthy subjects. Prog Neuro-Psychopharmacol Biol Psychiatry 28(1):9–104
64. Bremner I (1998) Manifestations of copper excess. Am J Clin Nutr 67(5 Suppl):1069S–1073S
65. Armutcu F, Ozerol E, Gurel A, Mehmet K, Vural H, Yakinci C, Akyol O (2004) Effect of long-term therapy with sodium valproate on nail and serum trace element status in epileptic children. Biol Trace Elem Res 102:1–10
66. Blair RE, Sombati S, Lawrence DC, McCay BD, DeLorenzo RJ (2004) Epileptogenesis causes acute and chronic increases in GABAA receptor endocytosis that contributes to the induction and maintenance of seizures in the hippocampal culture model of acquired epilepsy. J Pharmacol Exp Ther 310(3):871–880
67. Suh SM, Csima A, Fraser D (1971) Pathogenesis of hypocalcemia in magnesium depletion. Normal end-organ responsiveness to parathyroid hormone. J Clin Invest 50:2668
68. Anast CS, Mohs JM, Kaplan SL, Burns TW (1972) Interrelationship of magnesium metabolism and parathyroid function in man. Pediatr Res 6:350 (Abstr)
69. Leaver DD, Parkinson GB, Schneider KM (1987) Neurological consequences of magnesium deficiency: correlations with epilepsy. Clin Exp Pharmacol Physiol 14:361–370
70. Hossmann KA (1993) Disturbances of cerebral protein synthesis and ischemic cell death. Prog Brain Res 96:161–177
71. Natelson S, Miletich DJ, Seals CF et al (1979) Clinical biochemistry of epilepsy. I. Nature of the disease and a review of the chemical findings in epilepsy. Clin Chem 25(6):889–897
72. McEwan MJ, Espie CA, Metcalfe J (2004) A systematic review of the contribution of qualitative research to the study of quality of life in children and adolescents with epilepsy. Seizure 13:3–14
73. Shaheen Uzma, Prasad DKV, Vandana Sharma, Suryaprabha T, Ahuja YR, Jyothy A, Munshi A (2014) Significance of MDR1 gene polymorphism C3435T in predicting drug response in epilepsy. Epilepsy Res 108:251–256