Title: Coenzyme Q10 Insufficiency Contributes in Duration and Frequency of Seizures in Epileptic Patients

Running Title: Coq10 Deficiency Correlates With Clinical Manifestation Of Seizure

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Highlights

- CoQ10 deficiency plays a role in the pathogenesis of epileptic seizure (ES)
- Decrement of CoQ10 level in ES may be contributed to further frequency and duration of seizure.
- There is no association between the type of seizure and CoQ10 levels in epileptic patients.

Plain Language Summary

Decreasing of antioxidants plays an important role in pathogenesis of epilepsy. Coenzyme Q10 (CoQ10) is a strong endogenous antioxidant that protects body against several diseases, however, the impact of CoQ10 in epilepsy is not clear. We evaluated the serum concentration of CoQ10 to test if there is any relationship between CoQ10 levels and clinical manifestation of seizure. We found that the levels of CoQ10 in epileptic patients were significantly lower compared to healthy volunteers. We also found that the clinical manifestation of seizure (duration of epilepsy and seizure frequency) were negatively correlated with serum CoQ10 levels.

Abstract

Purpose: Oxidative stress has recently emerged as a possible mechanism in pathogenesis of epilepsy. Coenzyme Q10 (CoQ10) is a strong endogenous antioxidant that protects cells from lipid oxidation and reactive oxygen species (ROS) production, however, the impact of CoQ10 on seizure characteristics in epileptic patients is not clear.

Methods: The current study enrolled patients with the epileptic seizure (ES) to evaluate the serum concentration of CoQ10 with the aim to investigate whether a relationship exists between CoQ10 levels with duration, frequency and type of seizure.
Results: A total of 39 patients with epileptic seizure and 35 healthy controls were included in the study. The levels of CoQ10 in ES patients were significantly lower in comparison with healthy controls (11.99±5.93 vs. 16.48±4.20 P<0.001). We also found that the duration of epilepsy and seizure frequency was negatively correlated with serum CoQ10 levels.

Conclusion: These findings indicate that CoQ10 deficiency might substantially contribute with the clinical signs of epileptic patients.

Keywords: CoQ10, epileptic seizures, oxidative stress, clinical signs of seizure

Introduction

Perturbation in the antioxidants and oxidants equilibrium results in a phenomenon called oxidative stress which is recently suggested as a mechanism contributing to epilepsy (Sudha, Rao et al. 2001). The association between generation of free radicals and their scavengers by antioxidant systems has been established in epilepsy. Increase in formation of reactive oxygen species (ROS) has been viewed in animal model of epilepsy as well as in neurodegeneration observed in epileptic humans (Militão, Ferreira et al. 2010). Recent evidence shows that pro-oxidant/antioxidant imbalance can also increase the probability of seizure recurrence (Ercegovac, Jović et al. 2013). In addition, elevation in ROS production may lead to prolonged seizures and development of subsequent epilepsy (Martinc, Grabnar et al. 2012, Méndez-Armenta, Nava-Ruíz et al. 2014).

Coenzyme Q10 (known as coenzyme Q, ubidecarenone, and ubiquinone) is a lipophilic compound mainly located in mitochondrial membranes of all tissues cells, and acts as a crucial enzyme cofactor in the mitochondrial respiratory chain for synthesis of adenosine triphosphate (ATP) (Matthews, Yang et al. 1998). It also plays an important role as a potent antioxidant either directly by scavenging the free radicals or indirectly by regenerating other antioxidants, such as vitamin E (Matthews, Yang et al. 1998, Sattarinezhad, Shafaroodi et al. 2014).
Since disturbance in the activity of antioxidative defense mechanisms may be accounted for pathogenesis of epilepsy, and CoQ10 functions as a strong endogenous antioxidant, it is imperative to unravel the effects of CoQ10 in epileptic patients. While the detrimental effect of CoQ10 insufficiency in epilepsy has been shown in in vivo studies (de Sales Santos, de Freitas et al. 2010, Tawfik 2011), no human studies have addressed this issue. Therefore, the first aim of present study was to quantify the serum concentration of CoQ10, and the second aim was to figure out the clinical relevance of type, frequency, and duration of seizures with CoQ10 levels.

**Methods:**

**Study population**

This analytical cross-sectional study was performed in Long Term Monitoring (LTM) ward of the Loghman Hakim Hospital, Tehran, Iran. Medical ethics committee of Shahid Beheshti University of Medical Sciences approved the current study (ethics committee number: IR.SBMU.RETECH.REC.1396.411). All the subjects agreed and completed the written informed consent.

Case group included patients who were admitted to LTM ward of Loghman Hakim Hospital due to repeated epileptic seizures. Demographic and seizure characteristics such as type, frequency, duration and repetition as well as history of epileptic medications were collected through an interview with the patients. All the patients underwent at least 48 hours of continuous scalp EEG monitoring to capture enough habitual seizures. The epilepsy type was confirmed by an epileptologist based on ictal and interictal EEG findings and the seizures semiology. Patients with any other medical, neurological, psychiatric disorders and history of recent head trauma were excluded from the study. Comparison group consisted of age and gender-matched healthy volunteers with no history of seizure. Either patients or controls were
not included in study if they received antioxidant medications such as vitamin C, corticosteroids, and non-steroidal anti-inflammatory drugs (NSAIDs) within the past month.

**Blood collection and measurement of Coenzyme Q10 levels**

Venous blood samples were taken in all the patients within 24 hours of a habitual seizure. Five millilitre of venous blood was collected from each participant of the study and centrifuged at 4000 × g for 10 minutes (at 4°C). Then serum was immediately separated and aliquots were stored at -80ºC.

Serum CoQ10 levels were measured using the commercially available ELISA (enzyme linked immunosorbent assay) kit, according to the manufacturer’s instructions (ZB-13164S-H9648; ZellBio GmbH, Ulm, Germany). The serum CoQ10 levels were expressed as ng/mL. The intra-assay and inter-assay coefficients of variation were 10% and 12%, respectively.

**Statistical analysis**

We used the mean difference and 95% confidence interval for absolute difference of CoQ10 level (ng/ml) in the study groups. To support the normality of distribution, Kolmogorov–Smirnov test was used. Continues and categorical data were reported by mean ± standard deviation and number (percent), respectively. Independent sample *t* test and chi-square tests were applied to compare quantitative and categorical variables in the study groups. One-way analysis of variance (ANOVA) test was used to assess the level of CoQ10 in different types of epilepsy. Furthermore, multiple linear regression analysis was performed to explore the association between CoQ10 and epilepsy characteristics (duration, frequency, and type of seizure). Statistical analysis was performed using the SSPS version 16.0 (SPSS, Inc., Chicago, IL, USA). The statistical significance level was considered as *P* < 0.05.

**Results:**

Demographic and clinical data for the subjects included in the study are shown in table 1. A total of 39 patients with epileptic seizures (ES), 22 men and 17 women with a mean age of
29.79±11.89 years, were included in the study. Comparison group consisted of 35 healthy volunteers, 13 men and 22 women with 26.97±6.62 years on average. There was no significant difference between age and gender of two groups.

Table 1 shows the serum concentration of CoQ10 in ES patients compared to healthy subjects. Our finding revealed that the ES patients had significantly lower level of CoQ10 compared to the comparison group, P<0.001.

The association of study group, age and sex with serum level of CoQ10 was examined. There was a statistical significant positive association between the study group and level of CoQ10 in both crud (β: 4.49, P <0.001) and adjusted (β: 4.24, P < 0.001) models. As shown in table 2, the healthy group showed a 4.24 ng/ml increase of the CoQ10 concentration when adjusted for age and sex covariates. It means that observed positive significance association between study group and serum level of CoQ10 was not attenuated after adjusting for age and sex of study subjects (Table 2).

We also measured the association of seizure type (generalized seizures versus focal seizures) with serum concentration of CoQ10. Among the 39 recorded ES episodes, 28 attacks (71.8%) were focal seizures and 11 attacks (28.2%) were generalized convulsive seizures. No statistically significant correlation was found between type of seizure and serum CoQ10 level.

Regarding clinical features of epilepsy, we then assessed the relation between serum levels of CoQ10 and the frequency and duration of seizures using the Pearson correlation test. The mean seizure frequency was 11.92±21.21 attacks per month, and the epilepsy duration was 13.51±11.44 years. Interestingly, our results showed a negative significant correlation between duration of epilepsy and serum levels of CoQ10 (r: -0.37, P=0.02). The similar result was also detected when the correlation between seizure frequency and levels of CoQ10 was examined (r: -0.36, P=0.02).

Discussion
Oxidative stress triggered by diminished endogenous antioxidants or enhanced oxidants formation is latterly considered as a possible mechanism underlying epileptic seizures (Martinc, Grabnar et al. 2014). CoQ10 is a strong antioxidant which protects cells from oxidative damage through inhibiting certain enzymes involved in the formation of ROS and generating other antioxidants (El-ghoroury, Raslan et al. 2009, Bhardwaj and Kumar 2016). However, the impact of CoQ10 on seizure characteristics in ES patients is not clear. In this study, we provide novel insights into the role of CoQ10 insufficiency in pathophysiology of epileptic seizures. We demonstrate, for the first time, that serum concentration of CoQ10 decreases in ES patients. Our findings show that increasing the deficiency of CoQ10 results in more frequent and longer lasting epilepsy.

It seems that the endogenous antioxidants and repair capacity, which normally overcome the increased production of oxidants in cells, is reduced in ES patients. We thus assessed the serum levels of CoQ10, as an endogenous antioxidant, in these patients. Although baseline levels of CoQ10 could not be measured in the ES patients in our study, we found that their serum concentrations of CoQ10 were significantly lower than the control group. Decreased CoQ10 levels has also been shown to associate with various neurological diseases, including cerebral infarction, neurodegenerative processes, cerebral ataxia and many other brain disorders (Lamperti, Naini et al. 2003, Spindler, Beal et al. 2009, Ramezani, Sahraei et al. 2018, Simani, Ryan et al. 2018). CoQ10 deficiency could result in enhancement of electrons transport to oxygen, which leads to significant generation of superoxide anion (O$_2^-$) in mitochondria (Chew and Watts 2004). It then may cause damage to cellular components due to the elevation of ROS and reduction in ATP production; however, the effect of insufficient CoQ10 in the former is more prominent in pathogenesis of the diseases (Lalkovičová and Danielisová 2016, Milanlioglu, Aslan et al. 2016). On the other hand, an increase in free radicals and decrease of antioxidant level has been observed in the development of epilepsy (CENGIZ, YÜKSEL et al.
2000, Verrotti, Basciani et al. 2002). Therefore, it is not clear what might underlie the decline of antioxidants such as serum CoQ10. Taken together, it can be postulated that attenuated level of CoQ10 leads to significant rise in ROS production, resulting in oxidative stress and thus neuronal damage in an epileptic brain.

To determine the potential role of CoQ10 in the type of seizures, we then measured the correlation between these two variables. Based on our data, CoQ10 levels were not different in seizure types. This suggests that the type of epilepsy is unlikely to affect serum concentration of CoQ10 in different ways. This is in consistent with prior work showing that there is no correlation between the activity of antioxidant enzymes or oxidative stress and types of seizure (Yiş, Seçkin et al. 2009).

This study provides clear evidence that there was correlation between serum levels of CoQ10 and seizure frequency and duration of epilepsy. This may be related to free radical mediated damage or decrease in activity of antioxidants caused by the deficiency of CoQ10 which seen in ES patients (CENGIZ, YÜKSEL et al. 2000, Verrotti, Basciani et al. 2002). It has been shown that the risk of seizure recurrence increases with CoQ10 deficiency (Yiş, Seçkin et al. 2009). An absolute or relative deficiency in CoQ10 results in remarkable increase in transfer of electrons to molecular oxygen in mitochondria, leading to overproduction of superoxide free radicals (Chew and Watts 2004). The detrimental role of free radicals in seizures has been reported in rodent studies showing that antioxidants can reduce the oxidative stress markers in epileptic animals, accompanying with decrease of their seizure manifestations (Tan, Manchester et al. 1998, Gupta, Briyal et al. 2002, Mohanan and Yamamoto 2002, Barros, Xavier et al. 2007, Xavier, Barbosa et al. 2007).

Furthermore, activity of CoQ10, as an antioxidant scavenger, leads to inhibit lipid peroxidation (Mancuso, Orsucci et al. 2010). In this regard, findings from animal seizure models show that treatment of epileptic rats with CoQ10 exerts the neuroprotective effects by
removal of free radicals and reduction in lipid peroxidation levels and nitrite content, leading to ameliorate seizure severity (de Sales Santos, de Freitas et al. 2010, Tawfik 2011). It has also been demonstrated that pretreatment with CoQ10 during the acute phase of pilocarpine-induced seizures results in lipid peroxidation reduction and antioxidant factors elevation, leading to an overall decrease in oxidative stress (Santos, Tomé et al. 2009).

Therefore, based on our results, the negative association between seizure frequency and duration of epilepsy with CoQ10 may be related to free radical mediated damage triggered by deficiency in CoQ10.

In conclusion, our study demonstrated that there were significantly decreased levels of CoQ10 in ES patients. We also found that deficiency of CoQ10 dramatically exacerbates the clinical features of epilepsy. These results favorably suggest the development of novel methods of therapy, possibly including adjunctive antioxidant treatment. However, the study will need to be replicated in a larger sample of subjects.

**Ethical Considerations**

**Compliance with ethical guidelines**

This study was approved by Medical ethics committee of Shahid Beheshti University of Medical Sciences (ethics committee number: IR.SBMU.RETECH.REC.1396.411). Written informed consent was obtained from all the participants.

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**Conflict of interest**

None of the authors has any conflict of interest to disclose.

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