EVALUATION OF SERUM VITAMIN D LEVELS IN A GROUP OF EGYPTIAN CHILDREN WITH FEBRILE SEIZURES: CASE CONTROL STUDY

By

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ABSTRACT

Background: The role of vitamin D (vit. D) deficiency in different neurological problems is gaining popularity. While the role of vit. D in epilepsy is widely understood, its role in febrile seizures (FS) is less well understood, with only a limited amount of research available. The broad distribution of vit. D receptors can account for its beneficial role in epilepsy. We therefore sought in this study to check out the impact of vit. D deficiency in the development of FS.

Methods: A sample of 28 children with simple FS and 26 age and sex matched controls (who had febrile disease without seizures) were enrolled sequentially in the study. Venous samples for vit. D level, complete blood count, and other laboratory tests were obtained within 2 hours of FS, and the results for both groups were statistically analyzed.

Results: Regarding the levels of vit. D in the serum, no significant statistical difference existed between both groups (22.12 ± 21.97 vs 29.28 ± 14.83 ng/ml, P = 0.170, for convulsive and non-convulsive patients, respectively.). On the other hand, significant statistical differences existed between them, as regards the serum levels of Na, K, and platelets count (137.1 ± 2.37 vs 140.92 ± 7.45 meq/L, P = 0.013; 4.07 ± 0.38 vs 4.30 ± 0.32 mmol/L, P = 0.020; 260.07 ± 129.88 vs 330.93 ± 116.51 cell/μL, P = 0.040, for convulsive and non-convulsive patients, respectively.).

Conclusion: The association between the development of seizures in children with FS and vit. D deficiency was not proved statistically. However, they had a lower level than the control group.

Keywords: Febrile Seizures; vitamin D; Egyptian Children.
INTRODUCTION

Febrile seizure (FS) is amongst the most prevalent medical conditions, affecting 3% to 5% of children around the world (Smith et al., 2019) (Leung et al., 2018). FS could be considered when seizures occur with increased body temperature above 38°C without any central nervous system (CNS) affection or metabolic abnormalities in children aged from 6 to 60 months (Mosili et al., 2020). FS is classified into typical (simple) and atypical (complex) febrile seizures. Typical FS is mostly generalized tonic-clonic seizure that lasts 15 minutes or less and does not recur within 24 hours in children who are neurologically stable and without psychomotor abnormalities (American Academy of Pediatrics, 2011) (Fukuyama et al., 1996). Atypical FS is a focal or generalized seizure that lasts more than 15 minutes and/or recures on the same day. Moreover, it can be associated with neurological deficits or followed by Todd’s paralysis (Leung et al., 2018) (Knudsen, 2000).

FS is likely attributed to genetic predisposition in combination with incompletely understood environmental risk factors (Mosili et al., 2020) (Shi et al., 2012). After the 1st episode of FS, the rate of recurrence is 30%, whereas the rate of recurrence is 50% after the 2nd episode. Male gender, less than 1 year of age, atypical seizure type, fever of 38°C–39°C for less than one day, positive family history of FS or epilepsy, and low serum sodium are all increase the risk factors of recurrence (Mosili et al., 2020) (Smith et al., 2019).

On the other hand, scientists are still focusing on vitamin D (vit. D) and are trying to resolve related physiological controversies. Despite being linked to several health issues, including orthopedic, cardiovascular, metabolic, CNS, and autoimmune disorders, the harmful effects of vit. D deficiency remain under investigation (Zmijewski et al., 2019) (Peterlik & Cross, 2005). Due to the increased rate of growth in children and teenagers, vit. D deficiency is quite prevalent in early life, and it is considered one of the most common medical hazards worldwide, especially in low-income countries (Zmijewski et al., 2019) (Andiran et al., 2012).

While the role of vit. D deficiency is well known in epilepsy, its involvement in the FS is not yet well established, and
there is relatively little accessible literature about that. The large distribution of vit. D receptors in almost all human body tissues and cells may explain the role of its deficiency in epilepsy (Eyles et al., 2005). It is hypothesized that its role in the CNS could be conducted via calcemic and non-calcemic pathways (Stewart et al., 2010). The non-calcemic effect includes its action on the nuclear receptors to alter the gene expression inside the CNS (Ramagopalan et al., 2010).

There are many factors that have been proven to be associated with FS and its recurrence. However, many risk factors are still unknown and need to be investigated in order to improve control and prevent recurrence (Bhat et al., 2018) (Amiri et al., 2010). Therefore, we aimed in this study to assess the association between the development of FS in susceptible children and vit. D deficiency.

**PATIENTS AND METHODS**

An observational case–control study was conducted in El Hussein University hospital, Al-Azhar University for boys, Cairo, Egypt, during the period between September 2021 and December 2021.

**Ethical considerations:**

1. Informed written consent was obtained from the candidate or their legal guardians.
2. An approval by the local ethical committee was obtained before the study.
3. The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
4. All the data of the candidates and results of the study are confidential, and the candidates have the right to keep it.
5. The candidates have the right to withdraw from the study at any time.

**Sample size and sampling technique:**

We included all children aged from 6 to 60 months with typical FS that presented to our emergency rooms or outpatient clinics (28 children) during the duration of the study (convenient sample). Also, 26 child age and sex matched with them (presented with fever but without seizures), were sequentially enrolled as controls.
Exclusion criteria and case definition:

Any child with atypical febrile seizures, history of epilepsy, or chronic neurological disease was omitted from the study. We also excluded those with apparent malnutrition, renal, hepatic, or any metabolic disorder that could interfere with vit. D Metabolism. The American Academy of Pediatrics guidelines published in 1996 and revised in 2011 guided the diagnosis of simple FS (American Academy of Pediatrics, 2011). These guidelines still exist as the most accepted and applicable guidelines worldwide.

All candidates were subjected to: (a) detailed medical history taking, (b) comprehensive clinical examination, (c) blood samples had been obtained from all participants and the following parameters were measured, in addition to the vit. D level; complete blood count (CBC), serum creatinine (Cr), serum alkaline phosphatase (ALP), blood urea nitrogen (BUN), serum calcium (Ca), serum sodium (Na), serum potassium (K), serum phosphorous (P).

Venous sampling was used to obtain 4 ml of blood in EDTA vacutainers as well as 4 ml in plain vacutainers. It was left to clot before centrifugation at room temperature to get serum. Specimens were kept frozen at -20°C. The enzyme-linked immunosorbent test was performed to evaluate 25-OH vit. D using a marketably obtainable kit (Orgentec Diagnostika GmbH, Mainz, Germany). CBC including RBC indices was measured using automated cell counter. vit. D is considered normal if a 25(OH) vit. D ≥ 30 ng/ml (Holick et al., 2011).

Statistical analysis:

The statistical package SPSS (Statistical Package for the Social Sciences) version 25 was used to compute and list the data. Data was outlined using mean and standard deviation for quantitative data, while frequency (count) and relative frequency (percentage) were used for categorical data. A student t-test or other equivalent has been used to compare the quantitative variables between the two main groups. A Chi square ($x^2$) test was performed for comparing categorical data. A logistic regression analysis was used to predict the association between variables. The level of significance was considered when the P-value was lower than 0.05.
RESULTS

The 54 children who participated in the study were categorized as follows: group I, or case group, consists of the 28 children with FS; and group II, or control group, consists of the 26 children with fever but without seizure. The mean age of the group I was (15.87 ± 13.92) months, while the mean age of the group II was (15.96 ± 12.11) months. Among the children in group I, 13 (46.42%) were females, while group II contained 12 (46.15%) females without a significant difference between the groups as regards gender (P = 1.00) (Table 1). There was also no significant statistical difference in mean body temperature between both groups (38.85+0.55 °C vs 38.38+1.97 °C for groups I and II, respectively, P = 0.227). Furthermore, no differences were found between both groups in terms of birth mode (P= 0.99), breastfeeding (P=0.75), or the presence of comorbidities (P=0.14).

Table (1): Demographic criteria of the participants

<table>
<thead>
<tr>
<th></th>
<th>Patients with febrile seizures</th>
<th>Febrile patients without seizures</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>28</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD) (months)</td>
<td>15.87 ± 13.92</td>
<td>15.96 ± 12.11</td>
<td>0.980</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>12</td>
<td>1.000</td>
</tr>
<tr>
<td>Male</td>
<td>15</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>38.85 ± 0.5</td>
<td>38.38 ± 1.97</td>
<td>0.227</td>
</tr>
</tbody>
</table>
Among the patients with FS, the number of patients with a single attack of seizure was 23 (82.14%). Only 3 children were reported to have had more than 1 seizure episode. The history of seizures was unclear with the remaining 2 children. As regards the type of seizure, 27 (96.43%) children were proven to have developed generalized tonic-clonic seizures. The remaining child developed generalized tonic seizures. Only 2 children (7.14%) were reported to have a positive family history of seizures.

The results of laboratory tests of both groups showed that there was no significant statistical difference between cases and controls as regards the vit. D level (22.12 ± 21.97 vs 29.28 ±14.83 ng/ml, for convulsive and non-convulsive patients, respectively, P =0.170). The other laboratory data of candidates in both groups is shown in Table (2).

Table (2): The results of laboratory tests of both groups

<table>
<thead>
<tr>
<th></th>
<th>Patients with febrile seizures (n=28)</th>
<th>Febrile patients without seizures (n=26)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit. D (ng/ml)</td>
<td>22.12 ± 21.97</td>
<td>29.28 ±14.83</td>
<td>0.170</td>
</tr>
<tr>
<td>Hb (gm/dL)</td>
<td>10.91± 0.99</td>
<td>11.09 ± 1.24</td>
<td>0.557</td>
</tr>
<tr>
<td>WBC (cells/microL)</td>
<td>10.51 ± 4.94</td>
<td>11.31 ± 4.52</td>
<td>0.538</td>
</tr>
<tr>
<td>Platelet (cells/microL)</td>
<td>260.07 ± 129.88</td>
<td>330.93 ± 116.51</td>
<td>0.040</td>
</tr>
<tr>
<td>Sodium (meq/L)</td>
<td>137.1 ± 2.37</td>
<td>140.92 ± 7.45</td>
<td>0.013</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.07 ± 0.38</td>
<td>4.30 ± 0.32</td>
<td>0.020</td>
</tr>
<tr>
<td>ALP (unit/L)</td>
<td>530.92 ± 181.39</td>
<td>519.36 ± 170.6</td>
<td>0.811</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.59 ± 0.76</td>
<td>9.81 ± 0.81</td>
<td>0.308</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.10 ± 0.69</td>
<td>4.21 ± 0.65</td>
<td>0.550</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.52 ± 0.07</td>
<td>0.56 ± 0.19</td>
<td>0.303</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>22.05 ± 9.8</td>
<td>26.87 ± 13.99</td>
<td>0.146</td>
</tr>
<tr>
<td>Blood sugar (mg/dL)</td>
<td>113.23 ± 28.01</td>
<td>105.84 ± 33</td>
<td>0.378</td>
</tr>
</tbody>
</table>

(ng) nanogram, (ml) milliliter, (dL) Deciliter, (meq) milliequivalent, (L) liter, (mmol) millimole, (mg) milligram, (microL) microliter.
Additionally, when the included children were divided into infants (< 1 year) and older children (>1 year), no significant difference in vit. D levels could be detected (28.19 ± 17.14 vs 23.6 ± 21.23 ng/ml, respectively, p = 0.383). By comparing the vit. D level in children with FS aged < 1 year with those aged > 1 year, there was no difference between them (25.24 ± 21.02 vs 21.54 ± 23.56 ng/ml, respectively, p = 0.664).

Moreover, when comparing infants who had FS and those who did not develop seizures as regards the vit. D level, no statistical difference between both groups would be noticed (respectively 25.24 ± 21.02 vs 29.75 ± 15 ng/ml, p = 0.514). Similarly, no statistical difference could be observed between the vit. D level in older children (> 1 year) with and without FS (respectively, 21.54 ± 23.56 vs 26.04 ± 17.25 p =0.594). The previous data is shown in Table (3).

**Table (3): Vitamin D levels of the candidates according to their age**

<table>
<thead>
<tr>
<th></th>
<th>Infants (&lt;1 year)</th>
<th>Children (&gt;1 year)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with febrile seizures</td>
<td></td>
<td></td>
<td>0.664</td>
</tr>
<tr>
<td>Number</td>
<td>15 (53.6%)</td>
<td>13 (46.4%)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D level (ng/ml)</td>
<td>25.24 ± 21.02</td>
<td>21.54 ± 23.56</td>
<td></td>
</tr>
<tr>
<td>Febrile patients without seizures</td>
<td></td>
<td></td>
<td>0.563</td>
</tr>
<tr>
<td>Number</td>
<td>14 (53.8%)</td>
<td>12 (46.2%)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D level (ng/ml)</td>
<td>29.75 ± 15</td>
<td>26.04 ± 17.25</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.514</td>
<td>0.594</td>
<td></td>
</tr>
<tr>
<td>All Patients</td>
<td></td>
<td></td>
<td>0.383</td>
</tr>
<tr>
<td>Number</td>
<td>29 (53.7%)</td>
<td>25 (46.3%)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D level (ng/ml)</td>
<td>28.19 ± 17.14</td>
<td>23.6 ± 21.23</td>
<td></td>
</tr>
</tbody>
</table>
The regression analysis revealed no association between the vit. D level and the development of seizures in febrile children as the P-value was 0.098 and odd ratio was 1.338 when testing the possibility of seizure when the vit. D level was < 30 ng/ml (Table 4).

**Table (4): Logistic regression analysis for prediction of seizures in febrile children**

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>P value</th>
<th>OR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D &lt; 30 ng/ml</td>
<td>0.290</td>
<td>0.183</td>
<td>2.693</td>
<td>0.098</td>
<td>1.338</td>
<td>0.956 - 1.873</td>
</tr>
</tbody>
</table>

(OR) odd ratio, (CI) conference interval.

**DISCUSSION**

Despite, the increasing interest in the role of vit. D deficiency in a variety of neurological disorders, its role in the development of FS is not well established yet. This article looked at the levels of vit. D in the sera of 54 children presenting with fever who had or didn't have FS. Although children with FS had lower vit. D levels than children without seizures (22.12 ± 21.97 vs 29.28 ±14.83 ng/ml), significant variation between both groups didn’t exist (P = 0.170).

Moreover, higher vit. D levels were found in the sera of infants (< 1 year) than older children (either with FS or not), but the difference was not significant (28.19 ± 17.14 vs 23.6 ± 21.23 ng/ml, respectively, p = 0.383). This finding can be attributed to vit. D supplementation in infants.

Receptors of vit. D are widely distributed throughout the brain, impacting both non-calcemic and calcemic functions (Holló et al., 2012). The association between seizures and vit. D was initially aroused in 1974 when the authors noted that it could work inside the CNS as a neurotransmitter and had anticonvulsant activity (Holló et al., 2012) (Christiansen et al., 1974). Additionally, vit. D may help to boost the effectiveness of other neuroprotective drugs. On the other hand, some metabolic adjustments that can increase vit. D demand have been claimed to occur during febrile illness (Holló et al., 2012). As far as we know, there are very few clinical trials assessing and comparing levels of vit. D in the sera of children with FS and febrile children without FS (Heydarian et al., 2020) (Bhat et al., 2020).
Remarkably, we found that the mean vit. D level of all 54 candidates was lower than the normal range, and this was contrasting what Heydarian et al. reported in their recent research (Heydarian et al., 2020). Meanwhile, the literature is crowded with studies which proved that the vit. D level was decreased in the sera of children who had FS (Shariatpanahi et al., 2018) (Vuletić et al., 2016) (Tekin et al., 2014) (Mantadakis et al., 2012). This discrepancy may be due to many factors including geographical, genetic, habits, cultural, and dietary differences.

When compared to their controls, our FS candidates had a considerably reduced platelet count. These findings have been confirmed by several articles and studies (Hassan et al., 2021) (Liu et al., 2018) (Gontko-Romanowska et al., 2017). The notable decrease in platelet count in the FS patients may point to the quickness of platelet consumption and the severity of the inflammation in children who are liable to FS (Hassan et al., 2021). The undoubted reasons and pathways have not yet been completely discovered.

Our results showed that the serum Na and serum K are markedly lower in FS group than the control group, which agree with previous studies about the role of electrolyte disturbances in pathogenesis of FS (Deia et al., 2018) (Hawas et al., 2018) (Chiarelli et al., 1985).

The electrolytic disturbances, mainly hyponatremia (could be due to SIADH syndrome) may play a role in frequent relapses of FS (Chiarelli et al., 1985). The stability of neuronal cell membrane that prevents seizures, is impaired in hyponatremia, due to increased calcium influx, and facilitation of action potential which will cause seizure generation (Deia et al., 2018). Decrement of serum K level in animals and humans, leads to neurological abnormalities (Hawas et al., 2018).

CONCLUSION

In conclusion, the association between vit. D levels and the development of FS in children hadn't been proved; however, vit. D levels were found to be lower in children who had FS. Furthermore, no link between vit. D levels and the age of children in both FS patients and febrile patients without FS has been established. To deeply investigate the presence of this association, large sample correlation studies may be recommended.
Recommendations: More studies should be done to investigate the pathogenesis as well as risk factors of FS to prevent its occurrence and recurrence in susceptible children and infants.

Acknowledgments: We acknowledge all the participants in this research.

Limitations: Lack of sufficient number of previous research studies on the topic.

Authors Contribution:
Mohammed A. Hassan and Hussein Awad El Gharieb were responsible for medical assessment and data collection. Tahseen Samir Mohammed Yousef was responsible for statistical analysis. Hossam M. Farid El Zamek was responsible for laboratory assessment. All authors designed the research and wrote the manuscript.

Conflicts of interest: No conflicts of interest to be declared.

REFERENCES


تقديم: تقييم مستويات فيتامين د في مصل مجموعة من الأطفال المصريين المصابين بالتشتنجات الحرارية: دراسة الحالة والمراجع

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النتائج: فيما يتعلق بمستويات فيتامين د في مصل الدم، لم يكن هناك فرق إحصائي ذو دلالة بينهما. (P = 0.170). من ناحية أخرى، كانت هناك اختلافات إحصائية ذات دلالة كبيرة بينهما، فيما يتعلق بمستويات مصل الصوديوم والبوتاسيوم وعديد الصفائح الدموية. (P = 0.013، P=0.020، P=0.040).

الاستنتاج: لم يثبت إحصائياً الارتباط بين تطور نوبات التشنجات الحرارية لدى الأطفال المصابين بها وبين النقص في فيتامين د، بالرغم من أنه كان لديهم مستوى أقل من المجموعة الضابطة.