Epilepsy & Behavior 97 (2019) 60–66

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

# Serum NGF levels may be associated with intrauterine antiepileptic exposure-related developmental problems

Ali Karayağmurlu [a,*], Onur Burak Dursun [b], İbrahim Selçuk Esin [c], Murat Coşkun [a]

[a] Department of Child and Adolescent Psychiatry, Faculty of Medicine, Istanbul University, İstanbul, Turkey
[b] Department of Child and Adolescent Psychiatry, University of Health Sciences, International School of Medicine, Istanbul, Turkey
[c] Department of Child and Adolescent Psychiatry, Faculty of Medicine, Atatürk University, Erzurum, Turkey

ARTICLE INFO

*Article history:*
Received 29 January 2019
Revised 3 May 2019
Accepted 8 May 2019
Available online 7 June 2019

*Keywords:*
Epilepsy
Antiepileptic drugs
Neurodevelopmental outcomes
Nerve growth factor

ABSTRACT

*Objective:* It has been shown that maternal epilepsy and antiepileptic drug use during pregnancy have adverse developmental outcomes in children. The aim of this study was to investigate the developmental outcomes of maternal epilepsy and prenatal antiepileptic exposure. We also looked for the associations between serum levels of glial cell-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) and developmental outcomes.
*Methods:* This is a retrospective, nonrandomized, case-controlled study. Fifty-three children aged two to six years old with maternal epilepsy were included in the case group. Fifty-three age- and gender-matched children without maternal epilepsy were included in the control group. Developmental assessment was conducted using the Denver II Developmental Screening Test (DDST-II). Serum levels of NGF and GDNF were measured using an enzyme-linked immunosorbent assay (ELISA) kit.
*Results:* Multiple regression analysis revealed that prenatal antiepileptic exposure was significantly associated with lower global developmental scores ($B = -7.5$, confidence interval (CI): $-13.1$; $-1.9$, $p = 0.009$) while periconceptional folate use was associated with a reduced risk for adverse developmental outcomes ($B = 6.6$, CI: 0.91; 12.3, $p = 0.024$). Children with prenatal antiepileptic exposure are at increased risk for global developmental delay (GDD) particularly for language domain ($p = 0.018$). We found a statistically significant positive correlation between NGF levels and global developmental scores ($r = 0.302$, $p = 0.009$). Serum levels of GDNF in children with maternal epilepsy were significantly lower than the children without maternal epilepsy ($p = 0.025$).
*Conclusions:* Prenatal antiepileptic exposure was related with the increased risk of GDD while periconceptional folate use was related with lower risk. Clinicians should inform all women in reproductive age with epilepsy about the possible benefits and risks of antiepileptic drug use during a possible pregnancy. Periconceptional folate use has protective effect on child development, and all women on antiepileptic drugs should be encouraged for periconceptional folate use. Serum NGF levels may be a promising biomarker for monitoring global development delay in at-risk population.

© 2019 Elsevier Inc. All rights reserved.

## 1. Introduction

The prevalence of epilepsy in general population varies between 0.6 and 1.0% affecting one in every 200 pregnancies [1,2]. Data from the scientific literature show that the incidence of developmental problems is higher in children of mothers with epilepsy compared with the children of unaffected mothers. While many factors may contribute to the occurrence, prenatal antiepileptic exposure has been reported among common causes of developmental problems in children with maternal epilepsy [3].

There are many in vitro studies showing that antiepileptic drugs influence neuronal development [4,5]. However, in current practice, antiepileptic drugs are used frequently during pregnancy as many authors suggest that complications related to untreated seizure may be more severe than the potential harm resulting from antiepileptic drug use during pregnancy [6]. Clinical studies have examined the relationship between antiepileptic exposure during pregnancy and developmental problems in children but reported inconsistent results [3,7,8]. There is a shortage of clinical trials investigating the pathophysiology of prenatal antiepileptic exposure-related developmental problems in children. Meador et al. suggested that antiepileptic drugs may affect neuronal development by inducing neuronal apoptosis, thereby reducing the expression of neurotrophins [9]. An in vitro study also reported that antiepileptic drugs reduced the expression of neurotrophins [10].

* Corresponding author at: Child and Adolescent Psychiatrist, Istanbul University, Faculty of Medicine, Department of Child and Adolescent Psychiatry, İstanbul, Turkey.
*E-mail address:* dralikarayagmurlu@gmail.com (A. Karayağmurlu).

https://doi.org/10.1016/j.yebeh.2019.05.013
1525-5050/© 2019 Elsevier Inc. All rights reserved.

Considering the important roles of neurotrophic factors in each step of neuronal development, it would be rationale to investigate whether neurotrophic factors play a role in the pathophysiology of maternal epilepsy or intrauterine antiepileptic exposure-related developmental problems [11,12]. However, to the best of our knowledge, except for a few animal studies, there are no previous clinical study in this field. The aim of this study was to examine the relationships between maternal epilepsy, prenatal antiepileptic exposure, and developmental problems in children with particular focus on the possible role of nerve growth factor (NGF) and glial cell-derived neurotrophic factor (GDNF) in the pathophysiology of intrauterine antiepileptic exposure-related developmental problems.

## 2. Materials and methods

### 2.1. Participants

This study was conducted at the Erzurum Atatürk University Medical Faculty Hospital, Turkey between February 2015 and February 2016. Hospital medical records, 2009 to 2013 years, were used to define pregnant mothers with or without epilepsy during the pregnancy and their children aged two to six years. These mothers were reached by telephone, informed about the study, and invited to participate to the study. Children of the mothers with epilepsy with or without antiepileptic drug use during pregnancy were designed as case group. Age- and gender-matched children of the mothers without epilepsy or chronic medical illness during pregnancy were designed as control group. To ensure more accurate evaluation of epilepsy or intrauterine antiepileptic exposure-related developmental problems, particular attention was paid for confounding factors that could affect mental development in study sample. Therefore, exclusion criteria were defined as below for all participants:

* The use of any teratogenic drug except for antiepileptic drugs during pregnancy,
* History of smoking, alcohol, or substance use during pregnancy,
* Presence of chronic medical, neurological, or genetic diseases in the mothers or in children that could have affected child development on its own.
* Presence of intellectual disability in either parent.
* Presence of any acute infectious disease as evidenced by a C-reactive protein (CRP) value above 5 to avoid interfering the effect of inflammation on study parameters.

Finally, one hundred and six children were included in the study. The participants were divided into two groups, case and control groups, each consisting of 53 children. In order to evaluate developmental outcomes of epilepsy and prenatal antiepileptic exposure, the case group was divided in two subgroups. The first subgroup was composed of 19 subjects with maternal epilepsy during pregnancy without the use of antiepileptic medications and the second group with 34 subjects with prenatal antiepileptic exposure during pregnancy. Those mothers who did not use antiepileptic medications during pregnancy discontinued medication before or just after suspected or confirmed pregnancy within four weeks of conception. The subgroups of the case group and the control group are shown in Fig. 1.

### 2.2. Procedure

Mothers who accepted to take part in the study were invited to the hospital together with their children. They were given detailed information about the study, assessed for all inclusion and exclusion criteria, and asked for informed consent to take part in the study if they met all study criteria. Participating mothers and their children were evaluated for the study using sociodemographic data form and the Denver II Developmental Screening Test (DDST-II). Following developmental evaluation, 2–5 $cm^3$ blood samples were obtained from children after parental consent. Serum concentrations of GDNF and NGF were determined by enzyme-linked immunosorbent assay (ELISA) test. Serum levels of NGF and GDNF were measured with a Sun Red Biological commercial Human NGF and GDNF ELISA kit (Catalog No. DZE201122103) via a BIO-TEK/Power Wave XS micro plate reader. Ethical approval for this study was obtained from the Ataturk University Medical Faculty Ethical Committee on 19.02.2015 (No. 1/26).

### 2.3. Instruments

A sociodemographic form was developed by the authors to collect data on parental ages and education, history of physical or mental diseases in the parents, family income, planned or unexpected pregnancy, periconceptional folate use (5 mg/day), birth weight of the children, and history of chronic disease in children. Information was also obtained about seizures (i.e., types and numbers of seizures) during

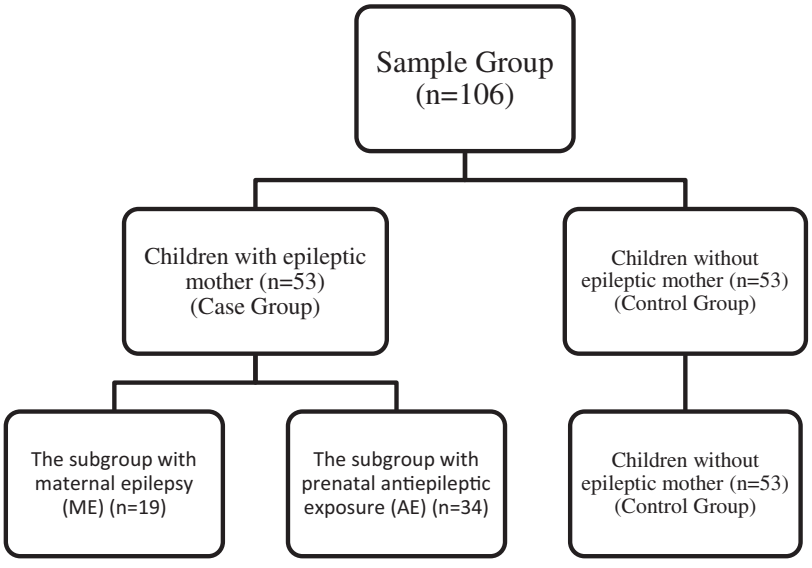


**Fig. 1.** The subgroups of the case group and control group.

pregnancy among mothers diagnosed with epilepsy and about antiepileptic medications used during pregnancy.

#### 2.3.1. Denver Developmental Screening Test II (DDST-II)

The DDST-II is used to evaluate the development of children in the 0–6 years age range. It consists of 134 items in four sections investigating the developmental domains of social, motor, communication, and daily living skills [13]. The Turkish reliability and validity study of the DDST-II was conducted by Anlar et al., and it has been used in several studies [14–17]. The developmental score is determined by the ratio of the number of performed items/the number of items that the patient should perform according to his/her age, and multiplying the result by 100. The development domain score and global development score were calculated together for all domains at statistical analysis [17].

### 2.4. Statistical analysis

All analyses were carried out on Statistical Package for the Social Sciences (SPSS) version 23 software. The relationship between qualitative variables was examined using the chi-square test. Continuous variables were tested for normal distribution using the Kolmogorov–Smirnov test [18]. Normally distributed continuous variables were compared using Student's t-test, one-way ANOVA, and the post hoc test. Some continuous distributions were not normal, and Spearman correlation analysis and the Mann Whitney U test were therefore applied. Tukey post hoc analyses were performed following one-way analysis of variance (ANOVA). Factors affecting global developmental scores were evaluated by composing a model using multiple linear regression analysis. Clinical and demographic variables associated with developmental problems including epilepsy, antiepileptic drug use during pregnancy, periconceptional folate use, premature birth, unplanned pregnancy, and low birth weight were computed (for all factors; absence: 0, presence: 1) in this model to assess independent variables of global developmental scores on DDST-II.

## 3. Results

The case and control groups exhibited similar sociodemographic characteristics. There was no statistically significant difference between the two groups in terms of age, gender, parental education level, and family income status. Preterm birth, low birth weight, history of unwanted pregnancy, and periconceptional folate use are confounding factors that increase the risk of developmental problems. There was no statistically significant difference between the two groups in any of these confounding factors. Prenatal antiepileptic exposure was present in 34 subjects including 18 subjects with carbamazepine (52.9%), 8 subjects with valproate (23.5%), 6 subjects with lamotrigine (17.6%), and 2 subjects with levetiracetam exposure (5.8%) during pregnancy. Of those mothers with epilepsy (n = 53), 19 subjects (35.8%) discontinued their medication before pregnancy. Of those mothers who discontinued medication before pregnancy (n = 19), nine of them (47.3%) discontinued antiepileptic therapy on their own decision without medical advice and ten mothers (52.7%) discontinued antiepileptic therapy under medical supervision. Seizures were focal onset in 31 (58.4%) of the 53 subjects diagnosed with epilepsy and generalized onset in 22 (41.6%). Twenty-three (74.2%) of the focal onset seizures were focal to bilateral tonic–clonic, and 8 (25.8%) were myoclonic. Seizures were generalized tonic–clonic in 17 (77.3%) of the subjects with generalized onset and myoclonic in 5 (22.7%). Thirty-nine (73.6%) subjects experienced five or fewer seizures during pregnancy while 14 (26.4%) experienced more than five seizures. There was no significant differences in global developmental scores according to the number of seizures during pregnancy (Independent sample t-test, t-score = 0.255, p = 0.800). Sociodemographic characteristics of the case and control groups are summarized in Table 1.

**Table 1**
Sociodemographic characteristics of the case and control groups.

| Variables | Case group (n = 53) | Control group (n = 53) | p-Value |
|---|---|---|---|
| Age (months) | 48.5 ± 19.5 | 50.3 ± 16.8 | 0.620 |
| Gender | | | 1.000 |
| Female | 30 (56.6%) | 30 (56.6%) | |
| Male | 23 (43.4%) | 23 (43.4%) | |
| Educational level of mother | | | 0.471 |
| Elementary school level | 33 (62.3%) | 36 (67.9%) | |
| Primary education | 8 (15.1%) | 4 (7.5%) | |
| High school or above | 12 (22.6%) | 13 (24.5%) | |
| Educational level of father | | | 0.087 |
| Elementary school level | 16 (30.2%) | 26 (49.2%) | |
| Primary education | 12 (22.6%) | 12 (22.6%) | |
| High school or above | 25 (47.2%) | 15 (28.3%) | |
| Periconceptional folate use | | | 0.196 |
| Yes | 12 (22.6%) | 18 (34.0%) | |
| No | 41 (77.4%) | 35 (66.0%) | |
| Unplanned pregnancy | | | 0.104 |
| Yes | 11 (20.8%) | 5 (9.4%) | |
| No | 42 (79.2%) | 48 (90.6%) | |
| Birth weight | | | 0.131 |
| 1500 mg or less | 1 (1.9%) | 2 (3.8%) | |
| 1500–2500 mg | 4 (7.7%) | 9 (17.0%) | |
| 2500–4500 mg | 46 (86.5%) | 41 (77.4%) | |
| 4500 mg or more | 1 (1.9%) | 1 (1.9%) | |
| Type of birth | | | 0.563 |
| Premature birth | 4 (7.7%) | 7 (13.2%) | |
| Mature birth | 47 (90.4%) | 46 (86.8%) | |
| Postmature birth | 1 (1.9%) | 0 (0%) | |

The DDST-II was applied to the sample group. A multiple linear regression model was composed to predict the factors affecting global development scores in the DDST-II. A statistically significant relationship was observed between the model and the development scores ($R^2$ = 0.171, p = 0.010). While maternal epilepsy itself was not associated with adverse developmental outcomes (B = 0.6, confidence interval (CI): −6.7; 6.8, p = 0.985), prenatal antiepileptic exposure was significantly associated with lower global developmental scores (B = −7.5, CI: −13.1; −1.9, p = 0.009). Further analysis revealed that while premature birth, unplanned pregnancy, and low birth weight were not associated with adverse developmental outcomes, periconceptional folate use was associated with a reduced risk for adverse developmental outcomes (B = 6.6, CI: 0.9; 12.3, p = 0.024). The multiple linear regression model is summarized in Table 2.

The global developmental scores of the maternal epilepsy, antiepileptic exposure, and control groups were compared in terms of periconceptional folate use. The global developmental scores of subjects with periconceptional folate use in the group with maternal epilepsy were 119.2 ± 14.0 (mean ± standard deviation (SD); n = 4), compared with 111.9 ± 12.3 (mean ± SD; n = 8) in the cases with no periconceptional folate use. The difference between the two groups was not statistically significant (Mann–Whitney U-test, z-score = −0.916, p = 0.360). The global developmental scores of subjects with periconceptional folate use in the antiepileptic exposure group were 107.5 ± 14.1 (mean ± SD; n = 8), compared with 101.9 ± 15.2

**Table 2**
Multiple linear regression analysis for predictors of global developmental scores.

| Variables | SE | Beta | T | p | OR | 95.0% CI for OR | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper |
| Epilepsy | 3.4 | 0.002 | 0.019 | 0.985 | 0.6 | −6.7 | 6.8 |
| Antiepileptic exposure | 2.8 | −0.284 | −2.658 | **0.009** | **−7.5** | −13.1 | −1.9 |
| Use of folic acid | 2.8 | 0.241 | 2.305 | **0.024** | **6.6** | 0.911 | 12.3 |
| Premature birth | 5.1 | 0.126 | 0.897 | 0.372 | 4.6 | −5.6 | 14.8 |
| Low birth weight | 4.8 | −0.199 | −1.386 | 0.169 | −6.6 | −16.2 | 2.9 |
| Unplanned pregnancy | 3.5 | −0.073 | −0.718 | 0.475 | −2.5 | −9.5 | 4.4 |

Notes: R = 0.414. $R^2$ = 0.171; p = 0.010. Bold data, p < 0.05 (significance). CI: confidence interval. SE: standard error; For all factors absence (0) → presence (1).

(mean ± SD; n = 26) in the cases with no periconceptional folate use. The difference between the groups was not statistically significant (Mann–Whitney U-test, z-score = −1.321, p = 0.187). In the control group, the global developmental scores of subjects with periconceptional folate use were 118.0 ± 14.6 (mean ± SD; n = 17), compared with 111.0 ± 8.9 (mean ± SD; n = 33) in those without periconceptional folate use. The difference between the two groups was statistically significant (Independent sample t-test, t-score = 2.083, p = 0.043). Prenatal antiepileptic exposure was associated with an increased risk for adverse developmental outcomes in global development in the DDST-II without significant differences across different antiepileptic medications including carbamazepine (mean ± SD = 105.3 ± 12.1; n = 17), valproate (mean ± SD = 95.3 ± 22.0; n = 7), and lamotrigine (mean ± SD = 110.4 ± 11.0; n = 6) [$F(2,28) = 1.44$, p = 0.485]. In order to determine which developmental domains were affected within the global developmental scores, the prenatal antiepileptic exposure subgroup was compared with the subgroup with maternal epilepsy without prenatal antiepileptic exposure and control group for each developmental domain. In the one-way ANOVA, there was a significant difference between the groups in the fine motor [$F(2,93) = 6.17$, p = 0.003] and language development scores [$F(2,93) = 4.16$, p = 0.018]. Post hoc analyses were performed on the global development, language development, fine motor development, gross motor development, and personal–social development domains. The global development, fine motor, and language development scores of the prenatal antiepileptic exposure subgroup were significantly lower than those of the subgroup with maternal epilepsy without prenatal antiepileptic exposure and the control group (p < 0.05) (Table 3).

Correlation analyses were applied to NGF and GDNF serum levels and DDST-II scores. Statistically significant positive correlation was observed between NGF serum levels and language (r = 0.291, p = 0.011) and personal–social development (r = 0.247, p = 0.033) scores on DDST-II. Positive correlation was observed between GDNF serum levels and personal–social development scores (r = 0.356, p = 0.002). Marginally significant correlation was found between GDNF levels and global development scores (r = 0.216, p = 0.063). The correlation analysis of DDST-II development scores with NGF and GDNF serum levels is summarized in Table 4.

Correlation analysis was conducted to examine the relationship between serum NGF levels and global developmental scores for each subgroup including children with maternal epilepsy, prenatal antiepileptic exposure, and control groups. We found a significantly positive correlation between serum NGF levels and global developmental scores in the subgroup of children with prenatal antiepileptic exposure (r = 0.468, p = 0.021). Fig. 2 shows a correlation scatter plot for global developmental scores and serum NGF levels for three subgroups.

Post hoc analyses were run between the case subgroups and control group to evaluate GDNF and NGF serum levels. We determined a statistically significant difference in GDNF serum levels between the subgroup with maternal epilepsy only (ME) and control group [$F(2,77) = 2.68$, p = 0.025]. Comparisons of serum levels of GDNF and NGF using ANOVA and Tukey's test between the case subgroups and control group are shown in Table 5.

**Table 3**
Comparison of the DDST-II scores using of ANOVA and Tukey's tests between the control group and subgroups.

| Variables | Group ME[a] (n = 16) | Group AE[a] (n = 30) | Group CG[a] (n = 50) | ANOVA | Comparison group | Post hoc p-value |
|---|---|---|---|---|---|---|
| Global development score | 113.7 ± 12.7 | 104.3 ± 14.4 | 113 ± 0.12 | **0.006** | ME vs AE | **0.047** |
| | | | | | ME vs CG | 0.995 |
| | | | | | AE vs CG | **0.007** |
| Language development score | 126.8 ± 28.3 | 105.5 ± 29.9 | 122.5 ± 28.3 | **0.018** | ME vs AE | **0.049** |
| | | | | | ME vs CG | 0.858 |
| | | | | | AE vs CG | **0.033** |
| Fine motor development score | 109.9 ± 11.5 | 104.3 ± 11.2 | 112.8 ± 9.7 | **0.003** | ME vs AE | 0.203 |
| | | | | | ME vs CG | 0.598 |
| | | | | | AE vs CG | **0.002** |
| Personal–social development score | 108.0 ± 12.5 | 102.9 ± 12.5 | 109.1 ± 11.3 | 0.081 | ME vs AE | 0.359 |
| | | | | | ME vs CG | 0.944 |
| | | | | | AE vs CG | 0.069 |
| Gross motor development score | 109.9 ± 13.3 | 106.1 ± 15.8 | 109.9 ± 11.9 | 0.448 | ME vs AE | 0.977 |
| | | | | | ME vs CG | 0.646 |
| | | | | | AE vs CG | 0.351 |

ME: subgroup with maternal epilepsy only; AE: subgroup with prenatal antiepileptic exposure and maternal epilepsy; CG: control group; Bold data, p < 0.05 (significance).
[a] Data presented as mean ± SD.

**Table 4**
The correlation analysis of DDST-II development scores with plasma levels of NGF and GDNF.

| | Correlation coefficient | p-Value |
|---|---|---|
| NGF — global development score | 0.302 | **0.009** |
| NGF — linguistic development score | 0.291 | **0.011** |
| NGF — personal–social development score | 0.247 | **0.033** |
| NGF — fine motor development score | 0.128 | 0.273 |
| NGF — gross motor development score | 0.206 | 0.077 |
| GDNF — global development score | 0.216 | 0.063 |
| GDNF — linguistic development score | 0.156 | 0.181 |
| GDNF — personal–social development score | 0.356 | **0.002** |
| GDNF — fine motor development score | −0.018 | 0.877 |
| GDNF — gross motor development score | 0.144 | 0.218 |

Bold data, p < 0.05 (significance).

## 4. Discussion

We investigated developmental outcomes of maternal epilepsy and prenatal antiepileptic exposure in an at-risk population. We also examined serum levels of NGF and GDNF as possible moderating factors for epilepsy or antiepileptic exposure-related developmental problems. As far as we know, this is the first study to examine the serum levels of neurotrophic factors in an at-risk group with maternal epilepsy and prenatal antiepileptic exposure.

### *4.1. Developmental outcomes of maternal epilepsy and prenatal antiepileptic exposure*

In our study, similar to previous studies, we did not find an association between maternal epilepsy and developmental problems. Banach et al. compared the longitudinal developmental outcomes of children with maternal epilepsy with or without prenatal antiepileptic exposure. They concluded that maternal epilepsy was not associated with adverse developmental outcomes. However, the authors also pointed out that the presence of less severe epilepsy in the group without antiepileptic exposure may be confusing [19]. It may be important to note that our study may suffer from the same confounding factor. In a similar study by Thomas et al., maternal epilepsy did not have adverse effects on mental and motor development [20].

In our study, prenatal antiepileptic exposure rather than maternal epilepsy was found to be associated with adverse global developmental outcomes, particularly in language and fine motor domains. These findings are in line with previous studies reporting language and motor development problems related with prenatal antiepileptic exposure. In the third year follow-up of the cohort study of the Neurodevelopmental Effects of Antiepileptic Drugs (NEAD) group, prenatal antiepileptic exposure with valproate and carbamazepine was found to exhibit dose-dependent adverse effects on verbal abilities. However, the difference had disappeared by the sixth year of follow-up, except for the dose-dependent effect of valproate [9]. Similarly, Gaily et al. observed significantly lower verbal IQ (Intelligence Quotient) scores in subjects with prenatal exposure to valproic acid [21]. In a prospective cohort study, Veiby et al. determined that prenatal antiepileptic exposure was associated with impaired-fine motor skills at six months of age. The

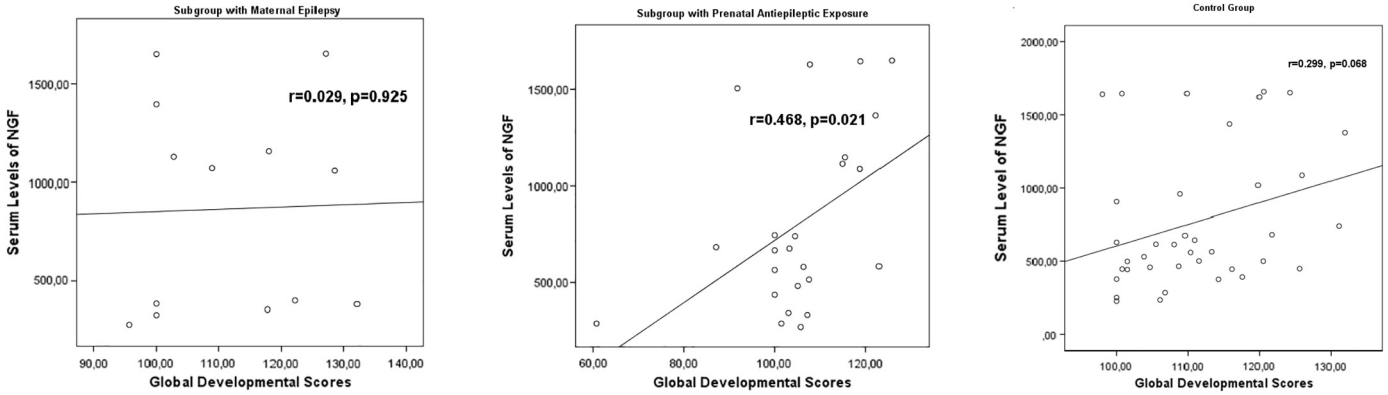


**Fig. 2.** The correlation scatter plot for serum NGF levels and global developmental scores in three subgroups.

impairment in fine motor skills had also persisted at eighteen months of age [22]. Similarly, Thomas et al. found that children with prenatal antiepileptic exposure had significantly lower motor development than those without prenatal antiepileptic exposure in children with maternal epilepsy [20]. Post hoc analysis of our findings showed that prenatal antiepileptic exposure adversely affected global development, especially language skills. Language is one of the most vulnerable abilities of the human brain [23]. Impairment in cognitive functions may adversely affect language acquisition, especially during early development [23].

Folate is an essential molecule that plays important roles in cell proliferation with a need of increased levels during pregnancy [24,25]. One hypothesis regarding the adverse neurodevelopmental outcomes of antiepileptic drugs is that they reduce the concentration of folate in the blood as supported by several animal studies [6,26–28]. However, there seems to be a lack of clinical studies on this topic. In a clinical study examining neurodevelopmental outcomes following prenatal antiepileptic exposure, the use of periconceptional folate was shown to have a significant positive effect on cognitive outcomes [9]. In our study, multiple linear regression analysis revealed that the use of periconceptional folate was associated with the lower risk of global developmental delay (GDD). This finding obtained from a retrospectively designed study may support the finding of the previous prospective study by Meador et al. [9].

Excitatory neuronal activity is important for normal brain development, and antiepileptic drugs may suppress this neuronal activity. Suppression of the neuronal activity by antiepileptic drugs adversely affects the formation of synapses, connectivity, and production of oligodendrocytes that may result in developmental problems [29,30]. Another possible explanation is that exposure of the immature brain to antiepileptic drugs may cause extensive neuronal apoptosis and reduce the expression of neurotrophic factors [30]. In this study, we aimed to investigate serum levels of NGF and GDNF in children with maternal epilepsy with or without prenatal antiepileptic exposure and found several important findings.

**Table 5**
Comparison of serum GDNF and NGF levels using ANOVA and Tukey's test between the control group and subgroups.

| Variables | Group ME[a] (n = 16) | Group AE[a] (n = 24) | Group CG[a] (n = 40) | ANOVA | Comparison group | Post hoc p-value |
|---|---|---|---|---|---|---|
| GDNF | 6.7 ± 4.4 | 8.4 ± 7.3 | 11.1 ± 7.3 | 0.075 | ME Vs AE | 0.659 |
| | | | | | ME Vs CG | **0.025** |
| | | | | | AE Vs CG | 0.330 |
| NGF | 780.5 ± 505.6 | 805.5 ± 465.8 | 756.6 ± 463.1 | 0.922 | ME Vs AE | 0.986 |
| | | | | | ME Vs CG | 0.985 |
| | | | | | AE Vs CG | 0.913 |

ME: subgroup with maternal epilepsy only; AE: subgroup with prenatal antiepileptic exposure and maternal epilepsy; CG: control group; Bold data, $p < 0.05$ (significance).
[a] Data presented as mean ± SD.

### 4.2. Relationships between NGF and GDNF levels and developmental scores

A better understanding of the mechanisms involved in neurodevelopmental disorders and the identification of molecules involved in their development are crucial in terms of early diagnosis and intervention. Although there have been major advances in neuroscience, insufficient biomarkers are available to guide clinicians regarding the diagnosis of neurodevelopmental disorders [31]. In our study, NGF and GDNF levels were also examined in terms of the hypothesis that maternal epilepsy and antiepileptic exposure may lead to neurodevelopmental problems via alterations in the level or functions of neurotrophic factors.

Although NGF is known to play an important role during the development of the central nervous system, it is still unclear whether it has any involvement in the emergence of neurodevelopmental disorders [32]. We observed a statistically significant positive correlation between plasma NGF levels and global development scores. This finding was consistent with other previous studies. Pinheiro et al. reported positive correlation between infants' developmental scores and NGF levels in mothers with postpartum affective disorders [33]. However, authors noted that a limitation of their study was that blood samples were not collected from the infants. In the present study, however, blood samples were collected from children. Similarly, Riikonen investigated whether neurotrophic factors play a role in the pathophysiology of Rett syndrome, a neurodevelopmental disorder characterized by GDD. Their results showed that the level of NGF in cerebrospinal fluid was significantly lower in Rett syndrome cases than the control group consisting of autism and other neuropediatric patients [34]. In another study, comparing infants with intrauterine growth retardation and healthy infants, levels of NGF were significantly higher in the healthy group, and plasma NGF levels were positively correlated with infant birth weight and percentiles [35]. The findings from current study may support the hypothesis of Meador et al. suggesting that adverse neurodevelopmental outcomes resulting from prenatal antiepileptic exposure might be associated with a decrease in the levels of neurotrophic factors [9]. We observed a significant positive correlation between plasma levels of NGF and developmental scores. Based on the results of our study, it may be argued that levels of NGF are related to global development. Further research with larger samples is needed to elicit a better understanding of mechanisms underlying developmental outcomes of prenatal antiepileptic exposure.

Glial cell-derived neurotrophic factor is a protective and restorative neurotrophic factor for neurons [36]. The risk of epilepsy is higher in children with maternal epilepsy than unaffected populations [37]. Jankowsky and Patterson suggested that GDNF plays an important

role in epilepsy, and GDNF upregulation may exhibit an antiepileptic protective effect [38]. Similarly, in their animal study, Jia et al. suggested that a low GDNF level may cause adverse neurodevelopmental outcomes in animals [39]. They concluded that increased GDNF expression (upregulation) is effective in preventing epilepsy and neuronal apoptosis [39]. In our study, we found that GDNF plasma levels were significantly lower in children with maternal epilepsy than the control group. This decrease was associated with epilepsy rather than prenatal antiepileptic exposure. In line with the previous studies, this finding from our study may support the hypothesis that GDNF levels may play a role in the etiopathogenesis of epilepsy [39,40]. However, to date, only one investigated the association between GDNF and developmental problems. Ibili et al. examined the DDST-II scores and GDNF levels of children with GDD at the time of diagnosis and 6 months after commencement of an intervention program for GDD. They reported a significant increase in both GDNF levels and DDST-II scores after the intervention program and suggested that GDNF might be related to GDD [17]. Together with the study by Ibili et al., our study is the second clinical study in this field reporting a marginally significant positive relationship between plasma GDNF levels and the global development score.

### 4.3. Strengths and limitations

The current study is one of the rare clinical biochemical studies conducted in an at-risk group with prenatal antiepileptic exposure and provides valuable information on the neurodevelopmental outcomes of prenatal antiepileptic exposure. However, some limitations also apply. The principal limitation was the relatively small sample size. Another limitation is that the at-risk group was not homogeneous in terms of prenatal antiepileptic exposure. The retrospective design and lack of randomization are other limitations, although these apply to all studies of antiepileptic drug exposure during pregnancy. In addition, the use of parental education level as a surrogate for parental IQ and the use of DDST-II that may not be a robust measure for developmental assessment may be other limitations.

### 4.4. Conclusion

Prenatal antiepileptic exposure may be related with developmental problems in children of mothers with epilepsy. Plasma NGF level may be a potential biomarker in diagnosing and follow-up of prenatal antiepileptic exposure-related developmental problems. Glial cell-derived neurotrophic factor levels may be lower in children with mothers with epilepsy supporting the link between GDNF and epilepsy. Periconceptional folate use may be protective against adverse developmental outcomes of prenatal antiepileptic exposure. This may be important as a protective measure in at-risk pregnancies.

## Acknowledgments

We would like to express our most sincere gratitude to the patients and families who agreed to participate in our study.

## Source of funding

The final manuscript has been reviewed and approved by all authors. No one of the authors has any kind of financial or institutional affiliations with pharmaceutical companies and state that they have no conflicts of interest. We all accept full responsibility for the cases. The paper had not been published elsewhere previously.

## Declaration of Competing Interest

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## References

[1] Brodie MJ, Dichter MA. Antiepileptic drugs. N Engl J Med 1996;334:168–75 https://doi.org/10.1056/NEJM199601183340308.
[2] De Bittencourt P, Adamolekum B, Bharucha N, Carpio A, Cossio O, Danesi M, et al. Epilepsy in the tropics: I. Epidemiology, socioeconomic risk factors, and etiology. Epilepsia 1996;37:1121–7 https://doi.org/10.1111/j.1528-1157.1996.tb01035.x.
[3] Bromley R, Weston J, Adab N, Greenhalgh J, Sanniti A, McKay AJ, et al. Treatment for epilepsy in pregnancy: neurodevelopmental outcomes in the child. Cochrane Database Syst Rev 2014;10:1–171. https://doi.org/10.1002/14651858.CD010236.pub2.
[4] Meador KJ, Gilliam FG, Kanner AM, Pellock JM. Cognitive and behavioral effects of antiepileptic drugs. Epilepsy Behav 2001;2:SS1–SS17 https://doi.org/10.1006/ebeh.2001.0235.
[5] Dansky LV, Finnell RH. Parental epilepsy, anticonvulsant drugs, and reproductive outcome: epidemiologic and experimental findings spanning three decades; 2: human studies. Reprod Toxicol 1991;5:301–35 https://doi.org/10.1016/0890-6238(91)90091-s.
[6] Meador KJ, Zupanc ML. Neurodevelopmental outcomes of children born to mothers with epilepsy. Cleve Clin J Med 2004;71(Suppl. 2):S38–41 https://doi.org/10.3949/ccjm.71.suppl_2.s38.
[7] Bromley RL, Mawer GE, Briggs M, Cheyne C, Clayton-Smith J, Garcia-Finana M, et al. The prevalence of neurodevelopmental disorders in children prenatally exposed to antiepileptic drugs. J Neurol Neurosurg Psychiatry 2013;84:637–43 https://doi.org/10.1136/jnnp-2012-304270.
[8] Cummings C, Stewart M, Stevenson M, Morrow J, Nelson J. Neurodevelopment of children exposed in utero to lamotrigine, sodium valproate and carbamazepine. Arch Dis Child 2011;96:643–7 https://doi.org/10.1136/adc.2009.176990.
[9] Meador KJ, Baker GA, Browning N, Cohen MJ, Clayton-Smith J, Kalayjian LA, et al. Foetal antiepileptic drug exposure and verbal versus non-verbal abilities at three years of age. Brain 2011;134:396–404 https://doi.org/10.1093/brain/awq352.
[10] Roullet F, Wollaston L, Foster J. Behavioral and molecular changes in the mouse in response to prenatal exposure to the anti-epileptic drug valproic acid. Neuroscience 2010;170:514–22 https://doi.org/10.1016/j.neuroscience.2010.06.069.
[11] Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 2001;24:677–736 https://doi.org/10.1146/annurev.neuro.24.1.677.
[12] Skaper SD. The neurotrophin family of neurotrophic factors: an overview. Methods Mol Biol 2012;846:1–12 https://doi.org/10.1007/978-1-61779-536-7_1.
[13] Frankenburg WK, Dodds J, Archer P, Shapiro H, Bresnick B. The Denver II: a major revision and restandardization of the Denver Developmental Screening Test. Pediatrics 1992;89:91–7.
[14] Anlar B, Yalaz K, Denver II. Gelişimsel Tarama Testi-Türk Çocuklarına Uyarlanması ve Standardizasyonu. Hacettepe Çocuk Nörolojisi Gelişimsel Tıp Araştırmaları Grubu. Ankara. s; 1996; l9–21.
[15] Soylu E, Soylu N, Polat C, Sakallıoğlu Ö, Uçur Ö, Bozdoğan G. Developmental delays in preschool children with adenotonsillar hypertrophy. Kulak Burun Bogaz Ihtis Derg 2016;26:126–34 https://doi.org/10.5606/kbbihtisas.2016.42724.
[16] Celikkiran S, Bozkurt H, Coskun M. Denver developmental test findings and their relationship with sociodemographic variables in a large community sample of 0–4-year-old children. Noro Psikiyatr Ars 2015;52:180–4 https://doi.org/10.5152/npa.2015.7230.
[17] Ucuz II, Dursun OB, Esin IS, Ozgeris FB, Kurt N, Kiziltunc A, et al. The relationship between vitamin D, autistic spectrum disorders, and cognitive development: do glial cell line-derived neurotrophic factor and nerve growth factor play a role in this relationship? Int J Dev Disabil 2015;61:222–30 https://doi.org/10.1179/2047387714Y.0000000054.
[18] Kolmogorov A. Sulla determinazione empirica di una lgge di distribuzione. Inst Ital Attuari 1933;4:83–91 Giorn.
[19] Banach R, Boskovic R, Einarson T, Koren G. Long-term developmental outcome of children of women with epilepsy, unexposed or exposed prenatally to antiepileptic drugs: a meta-analysis of cohort studies. Drug Saf 2010;33:73–9 https://doi.org/10.2165/11317640-000000000-00000.
[20] Thomas SV, Ajaykumar B, Sindhu K, Nair MK, George B, Sarma PS. Motor and mental development of infants exposed to antiepileptic drugs in utero. Epilepsy Behav 2008;13:229–36 https://doi.org/10.1016/j.yebeh.2008.01.010.
[21] Gaily E, Kantola-Sorsa E, Hiilesmaa V, Isoaho M, Matila R, Kotila M, et al. Normal intelligence in children with prenatal exposure to carbamazepine. Neurology 2004;62:28–32 https://doi.org/10.1212/wnl.62.1.28.
[22] Veiby G, Engelsen BA, Gilhus NE. Early child development and exposure to antiepileptic drugs prenatally and through breastfeeding: a prospective cohort study on children of women with epilepsy. JAMA Neurol 2013;70:1367–74 https://doi.org/10.1001/jamaneurol.2013.4290.
[23] Simmons ES, Paul R. Disorders of communication. In: Martin A, Bloch MH, Volkmar FR, editors. Lewıs's Chıld and adolescent Psychıatry. 5th ed. China: Wolters Kluwer; 2018.
[24] Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr 2012;3:21–38 https://doi.org/10.3945/an.111.000992.
[25] Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. Am J Clin Nutr 1996;63:520–5 https://doi.org/10.1093/ajcn/63.4.520.
[26] Mx Zhu, Ss Zhou. Reduction of the teratogenic effects of phenytoin by folic acid and a mixture of folic acid, vitamins, and amino acids: a preliminary trial. Epilepsia 1989;30:246–51 https://doi.org/10.1111/j.1528-1157.1989.tb05462.x.
[27] Elmazar MM, Thiel R, Nau H. Effect of supplementation with folinic acid, vitamin B6, and vitamin B12 on valproic acid-induced teratogenesis in mice. Fundam Appl Toxicol 1992;18:389–94 https://doi.org/10.1093/toxsci/18.3.389.

[28] Kim SH, Lee IC, Baek HS, Lim JH, Moon C, Shin DH, et al. Dose–response effects of diphenylhydantoin on pregnant dams and embryo-fetal development in rats. Birth Defects Res B Dev Reprod Toxicol 2012;95:337–45 https://doi.org/10.1002/bdrb.21022.
[29] Bergles DE, Roberts JDB, Somogyi P, Jahr CE. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 2000;405:187–91 https://doi.org/10.1038/35012083.
[30] Turski CA, Ikonomidou C. Neuropathological sequelae of developmental exposure to antiepileptic and anesthetic drugs. Front Neurol 2012;3:120–7 https://doi.org/10.3389/fneur.2012.00120.
[31] Mullin AP, Gokhale A, Moreno-De-Luca A, Sanyal S, Waddington JL, Faundez V. Neurodevelopmental disorders: mechanisms and boundary definitions from genomes, interactomes and proteomes. Transl Psychiatry 2013;3:e329. https://doi.org/10.1038/tp.2013.108.
[32] Dhobale M. Neurotrophins: role in adverse pregnancy outcome. Int J Dev Neurosci 2014;37:8–14 https://doi.org/10.1016/j.ijdevneu.2014.06.005.
[33] Pinheiro KA, Pinheiro RT, Coelho FM, da Silva RA, Quevedo LA, Schwanz CC, et al. Serum NGF, BDNF and IL-6 levels in postpartum mothers as predictors of infant development: the influence of affective disorders. PLoS One 2014;9:e94581 https://doi.org/10.1371/journal.pone.0094581.
[34] Riikonen R. Neurotrophic factors in the pathogenesis of Rett syndrome. J Child Neurol 2003;18:693–7 https://doi.org/10.1177/08830738030180101101.
[35] Malamitsi-Puchner A, Nikolaou KE, Economou E, Boutsikou M, Boutsikou T, Kyriakakou M, et al. Intrauterine growth restriction and circulating neurotrophin levels at term. Early Hum Dev 2007;83:465–9 https://doi.org/10.1016/j.earlhumdev.2006.09.001.
[36] Pascual A, Hidalgo-Figueroa M, Piruat JI, Pintado CO, Gomez-Diaz R, Lopez-Barneo J. Absolute requirement of GDNF for adult catecholaminergic neuron survival. Nat Neurosci 2008;11:755–61 https://doi.org/10.1038/nn.2136.
[37] Hardies K, Weckhuysen S, De Jonghe P, Suls A. Lessons learned from gene identification studies in Mendelian epilepsy disorders. Eur J Hum Genet 2016;24:961–7 https://doi.org/10.1038/ejhg.2015.251.
[38] Jankowsky JL, Patterson PH. The role of cytokines and growth factors in seizures and their sequelae. Prog Neurobiol 2001;63:125–49 https://doi.org/10.1016/s0301-0082(00)00022-8.
[39] Jia C, Han S, Wei L, Dang X, Niu Q, Chen M, et al. Protective effect of compound Danshen (*Salvia miltiorrhiza*) dripping pills alone and in combination with carbamazepine on kainic acid-induced temporal lobe epilepsy and cognitive impairment in rats. Pharm Biol 2018;56:217–24 https://doi.org/10.1080/13880209.2018.1432665.
[40] Duveau V, Fritschy JM. PSA-NCAM-dependent GDNF signaling limits neurodegeneration and epileptogenesis in temporal lobe epilepsy. Eur J Neurosci 2010;32:89–98 https://doi.org/10.1111/j.1460-9568.2010.07272.x.