RESEARCH ARTICLE

ASSOCIATION OF EEG ABNORMALITY AND DEVELOPMENTAL DELAY IN PHENYLKETONURIA (PKU): AN ANALYTIC HISTORICAL CASE-CONTROL STUDY

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Abstract

Objective

Electroencephalogram (EEG) is an easy and non invasive evaluation method for diagnosis and early prognosis in children. Our aim was to assess the association between EEG and the patients' Developmental Quotient (DQ) level in phenylketonuria.

Materials & Methods

In this study, 94 PKU patients (45 boys, 49 girls; mean age: 8.5 ± 6.2 years) who were diagnosed through newborn screening tests or later were followed. PKU was confirmed with a serum phenylalanine concentration above 6 mg/dl in untreated newborns. The patients were matched in gender, age and phenylalanine level. The ASQ (Age and Stage Questionnaire) was used for evaluation of the developmental stage of the case (abnormal EEG) and control (normal EEG) groups and the Wechsler Intelligence Scale was used to assess cognitive and intellectual abilities. Finally, one way ANOVA and chi square tests were used for analysis and $P< 0.05$ was considered significant.

Results

The case group consisted of 63 patients (67%) with abnormal EEGs and the control group consisted of 31 patients (33%) with normal EEGs. In patients with abnormal EEGs, 34 (53%) had mild, nine (14%) had moderate and 20 (33%) had severe EEG changes. Distribution of high and low DQ levels in these three groups show significant difference (p=0.001). Distribution of DQ level in the abnormal and normal EEG patients showed a significant difference (p=0.001).

Conclusion

There was no significant difference between phenylalanine level in case and control groups; therefore, EEG findings may affect patients' developmental scores despite a normal phenylalanine level in PKU patients.

Keywords: ASQ, Wechsler Intelligent Test, DQ, EEG, PKU

Introduction

Since the normal function of the brain is not fully known, most types of mental retardation have not been identified metabolically and anatomically. Although the principal biochemical defect is obvious in disorders like phenylketonuria (PKU) and other similar disorders, the exact basis for brain dysfunction is not definite (1). Developmental delay is a term that needs to be clarified. Mental retardation and brain damage are still used commonly, but they bring in mind children who are not able

to learn. Developmental delay does not cause complete inability and the children with this problem can learn, although they need much more time to accomplish it. The Developmental Disabilities Act of November 2000 defines developmental disabilities as ''a severe, chronic disability in an individual five years of age or older" with the following characteristics:

1. A mental or physical defect or both are the reason
2. It manifests before 22 years of age
3. It may continue imprecisely
4. It limits three or more of the mentioned activities; namely, self-care, receptive and expressive language, learning, mobility, self-direction, capacity for independent living, and economic self-sufficiency.
5. It demonstrates special, interdisciplinary, generic support or other services specifically organized.

Low intelligence test scores, poor academic functions and adaptive skill defects are indicative of developmental delay. Motor developmental delay in receptive and expressive language skills is usually present, too. Genetic defects, such as phenylketonuria (PKU) or "inborn errors of metabolism" which are a single-gene disorder, are the primary etiology for developmental delay in school-aged children. They cause significant developmental delay by twelve months of age and mental retardation before starting school (2). The ASQ is a self-report tool that produces scores for bad and good occurrences using three internal versus external, stable versus unstable, and global versus specific causes. It presents 12 half good half bad theoretical events, in which the test-taker has to note the one main reason of each happening and then give a scale according to the 7-point continuum for each of the three internal versus external, stable versus unstable, and global versus specific causes. The ASQ has been proven to be a predictor of depression, physical health, and achievement in different fields (educational, job and physical exercise). It takes about 20 minutes to complete this questionnaire with no time limitation. The parents should complete these questionnaires when a child is 4, 6, 8, 12, 16, 18, 20, 22, 24, 27, 30, 33, 36, 42, 48, 54, and 60 months of age (3). The ASQ materials consist of 19 reproducible master questionnaires; 19 reproducible, age-appropriate scoring and data summary sheets; and the user's guide. Instructions for scoring questionnaires, sample letters to parents, agencies, and service providers, and activities sheets for parents that correspond to the ASQ age intervals are also included in this user's guide. A supplementary videotape, The Ages & Stages Questionnaires on a Home Visit, (Farrell & Potter, 1995) describes how to use the questionnaires (4). The outcome measures after birth are developmental quotients (DQs) or intelligence quotients (IQs) or both (5), the McCarthy Scales of Children's Abilities [for General Cognitive Index (GCI)] (6), and the Weschler Intelligence Scale for Children (for IQ) (7).

The Wechsler Intelligence Scales are a set of standardized tests for assessment of cognitive and intellectual proficiency in children and adults. The Wechsler Intelligence Scales for Children (regular, revised, and third edition) and Wechsler Preschool and Primary Scale of Intelligence are used to detect learning disabilities and developmental defects, for recognizing talents and following intellectual development and also as tools in school placement. For occupational proficiency, intellectual ability in the classroom, and determining organic issues, the Wechsler Adult Intelligence Scales (regular and revised) are employed. These two Wechsler Scales are often considered in neuropsychological testing for brain function evaluation in cases with neurological defects (8). The developmental delay in the cerebral cortex is one of the characteristic pathologic changes in untreated phenylketonuria patients who are characterized by increased phenylalanine (Phe) in the plasma causing mental retardation with yet unknown underlying mechanisms (9).

Deficiency in a hepatic enzyme called phenylalanine hydroxylase(PAH) due to an autosomal recessive metabolic genetic disorder is the characteristic of PKU (10). This enzyme is required to change the amino acid phenylalanine ('Phe') to the amino acid tyrosine. In PAH deficiency, phenylalanine increases, then transforms into phenylpyruvate and consequently becomes detectable in the urine (11). Based on blood Phe concentrations, PAH deficiency is classified into classic PKU when the Phe level is higher than 1200 μmol/L, mild PKU when the Phe level is higher than 600 but below 1200 μmol/L and mild HPA, when the blood Phe is higher than the upper reference limit, but below 600 μmol/L (12). EEG is an effortless, non invasive cerebral investigation, and also a useful tool for diagnosis and early prognosis in

newborns. EEG may detect focal lesions or specific etiology in full-term newborns with abnormal clinical signs. EEG background activity and sleep organization have a high prognostic value. EEG recording over a long period is able to detect seizures, with or without clinical manifestations, and to discriminate them from paroxysmal non epileptic movements. The EEG should therefore be recorded at the beginning of the first symptoms and, if possible, before any seizure treatment. EEG background activity is categorized as normal, abnormal (type A and type B discontinuous and hyperactive rapid tracing) or highly abnormal (inactive, paroxysmal, low voltage plus theta tracing) in the neonatal prognostic tool system (13).

Materials & Methods

In this case-control study which was carried out from 2009 to 2010, 94 PKU patients (45 boys, 49 girls; mean age, 8.5 ±6.2 years) who had been admitted to Mofid Children's Hospital, Tehran, were enrolled. These patients were diagnosed through newborn screening tests or were followed later in the disease process at the hospital. We considered a newborn to have PKU if the serum phenylalanine concentration was above 6 mg/dl when untreated (14).

The patients were matched in gender, age and phenylalanine level. We divided the patients into two case and control groups, in which the case group included patients who had abnormal EEGs and the control group was formed by those with normal EEGs.

The patients' age at the time of evaluation ranged largely from 1 month to 23 years. The diagnosis of classic PKU was made on the basis of a serum phenylalanine (Phe) level higher than 1200 mmol/l, clinical manifestations and urine pterin analysis, excluding atypical PKU. An informed consent was obtained from parents or the legal guardian. Age, gender and family history findings were recorded for all cases. A pediatric neurologist took the history of seizure in all cases and recorded positive findings, so all patients who had recent seizures were excluded and only those who were seizure-free for at least three months prior to the study were enrolled. Blood samples were collected from all participants for measuring serum phenylalanine to match serum phenylalanine in patients as a constant factor. All patients were referred for EEG recording with sleep deprivation in Mofid Children's Hospital. Based on EEG findings, we divided patients into the case group (children with abnormal EEGs) and the control group (children with normal EEGs). We evaluated the developmental stage of the two groups with the ASQ questionnaire. The Wechsler Intelligence Scales were used to evaluate cognitive and intellectual abilities in children. Finally, one way ANOVA and chi square tests were used for comparison of data between the patients in the two groups and $p< 0.05$ was considered significant.

Results

We studied 94 children (45 boys, 49 girls) with PKU. The mean age of the patients was (8.5 ±6.2) years. Of these patients, 43% (45 patients) had seizures and 47% (51 patients) were clinically seizure-free. In the case group, there were 63 patients (67%) whose EEG was abnormal and the control group consisted of 31 patients (37%) who had a normal EEG. In patients with an abnormal EEG, 34 patients (53%) had mild changes, nine patients (14%) had moderate changes and 20 patients (33%) had severe EEG changes. The phenylalanine level ranged from 8 mg/dL to 50 mg/dL (mean: 18.88 mg/dL±7.76) at diagnosis and from 0.4mg/dL to 18mg/dL (mean: 7.4mg/dL± 4.8) at the time of evaluation. There was no significant difference between phenylalanine levels in the case and control groups and there was no significant difference between the two genders regarding abnormal EEG findings in these patients (Table 1). Table 2 shows the distribution of DQ level in all patients. Seventy-five patients (79.8%) had a DQ lower than 90 (Table 2) (Figure 1).

There was no significant difference in age between the two groups of DQ levels (above and below 90) ($p=0.2$). There was no significant difference in DQ levels between girls and boys ($p=0.7$). Table 3 shows mean head circumference (HC) in the groups of high and low DQ level. There was a significant difference between HC and DQ level in PKU patients ($p=0.04$).

Table 4 shows mean phenylalanine level in PKU patients with normal and abnormal EEGs at the time of diagnosis. Statistical analysis showed no significant difference in mean phenylalanine level between these two groups of patients ($p>0.05$).

Distribution of DQ level in the case and control groups (abnormal and normal EEG patients) showed a significant difference; i.e. an abnormal EEG was associated with a higher percentage of low DQ levels (90.3% vs. 61.3%; p=0.001) (Table5).

In patients with abnormal EEGs, patients were categorized into three classes according to the severity of the abnormal EEG; therefore, we had three groups of mild, moderate and severe among the cases. Distribution of high and low DQ levels in these three groups showed a significant difference (p=0.001) (Table 6).

Table 1: Distribution of EEG findings based on gender in all patients

Gender \ EEG Findings	Normal (control)	Abnormal (case)
Girls	17(34.7%)	32(65.3%)
Boys	14(31.1%)	31(68.9%)

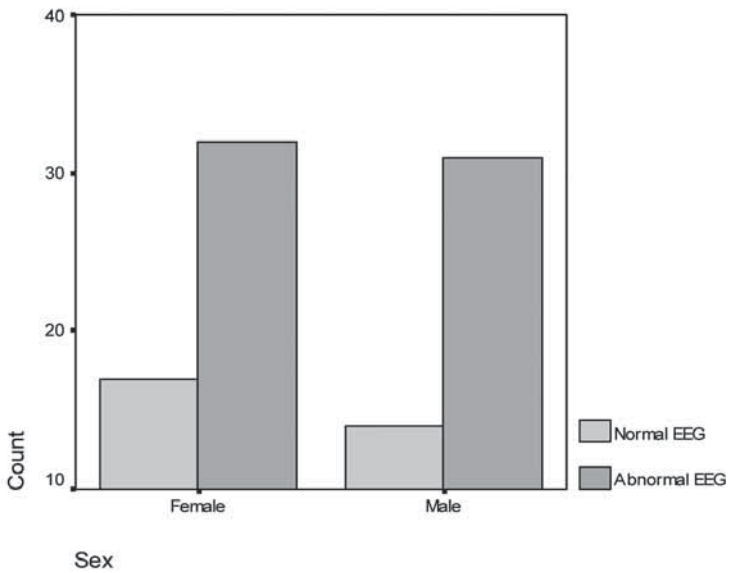

Fig 1. Distribution of normal and abnormal EEGs in girls and boys

Table 2: Distribution of DQ level in all patients

DQ	Frequency	Percent
<90	75	79.8
≥90	19	20.2
Total	94	100.0

Table 3: Mean head circumference in patients with high and low DQ levels at birth and at the time of evaluation

DQ Level	Mean±SD of HC	
	At birth	At evaluation
<90	34.7 ±1.5	48.8±4.8
≥90	34.6±1.7	47.5±6.4

Table 4: Mean phenylalanine level in normal and abnormal EEG groups at the time of diagnosis and evaluation

EEG finding	Mean of Phenylalanine level ± SD	
	At Diagnosis	At evaluation
Normal	21.06±10.2	7.48±4.4
Abnormal	17.71±5.8	7.31±5

Table 5: Distribution of high and low DQ levels in normal and abnormal EEGs

	DQ<90 N(%)	DQ≥90 N(%)
Abnormal EEG	56(90.3%)	6(9.7%)
Normal EEG	19(61.3%)	12(38.7%)

Table 6: Association between severity of abnormal EEGs and level of DQ in PKU patients

Severity of abnormal EEG	DQ<90	DQ≥90
Mild	29(85.3%)	5(14.7%)
Moderate	9(100%)	0
Severe	18(94.7%)	1(5.3%)

Discussion

We studied 94 children (45 boys, 49 girls) with PKU. In the case group, there were 63 patients (67%) whose EEGs were abnormal and in the control group, we had 31 patients (37%) with a normal EEG. In patients with an abnormal EEG, 34 patients (53%) had mild changes, nine patients (14%) had moderate changes and 20 patients (33%) had severe EEG changes. There was no significant difference in phenylalanine level between case and control groups. Distribution of DQ level in the case and control groups (abnormal and normal EEG patients) showed a significant difference; i.e. an abnormal EEG was associated with a higher percentage of low DQ levels (90.3% vs. 61.3%; p=0.001) (Table5). In a review of literature, clinical EEG findings and biochemical data were recorded in 10 children with classical PKU and five children with variant forms of phenylalanine increase during the first year of life. A semi quantitative evaluation

of the EEG showed a high correlation between epileptic form abnormalities and phenylalanine blood levels in the first 90 days of life and therefore with the delay before dietary therapy in PKU children.

Although performed on a limited population, such an approach may indicate an additional non-computerized EEG tool for the clinical management of hyper phenylalanine, and may subsequently suggest some criteria for neurophysiological risk evaluation during the first year of life (15). In our study, we matched phenylalanine level in all patients but failed to show the relationship between EEG findings and phenylalanine levels; however, there was an overall significant relationship between EEG findings and Phe level in all patients, which is similar to this study.

In a study conducted by Donker et al., the effect of dietary reintroduction of phenylalanine was evaluated on the EEG of six PKU patients who were on treatment. Patients received equally divided daily loads of 100 or 150 mg L-phenylalanine/kg over the meals. In the computerized spectral EEG analysis, the following EEG changes were detected: (1) activity appearance in the low frequency band (2-5 c/sec), (2) dominant rhythm frequency changes, (3) synchronous degree change between identical frequencies, happening in different derivations. As the blood level of phenylalanine increased, the EEG changes intensified and when the phenylalanine administration was discontinued, the EEG changes reversed, indicating that the degree of intoxication caused by phenylalanine or its metabolites was demonstrated in EEG abnormalities. It is believed that discontinuing the diet in PKU may be concluded based on the EEG data (16). In our study, the case group consisted of 63 patients (67%) who had an abnormal EEG and the control group consisted of 31 patients (37%) who had a normal EEG. In patients with EEG abnormality, 34 patients (53%) had mild changes, nine patients (14%) had moderate changes and 20 patients (33%) had severe EEG changes. In this study, we could not find any correlations between the level of phenylalanine and EEG findings, because we matched phenylalanine in the two groups, but there was a significant relationship between EEG findings and PHE level in the case group.

Pietz et al studied 34 children with phenylketonuria (PKU) who were treated early. They determined the prognostic value of age on the diet in the first 3 months of life and the quality of dietary treatment via following IQ closely and evaluating the EEG. IQ scores were normal from the 4^{th}-15^{th} years of life. Timing of diet onset had no effect on the IQ. A "strict" dietary control resulted in a significantly higher IQ compared to a "loose" control. In a 2-year interval, EEGs of 154 children recorded in the 10/20 system, awake with eyes closed, which were conventionally evaluated, showed alpha-activity development to be normal while beta-activity was enhanced. In children with PKU, abnormal EEG findings such as general slowing and generalized paroxysmal activity (GPA) with or without spikes were seen more frequently compared to the control group. Focal abnormalities were the exception, which were seen more in the control group. Aging increased EEG abnormalities independent of IQ development. EEG abnormalities showed no relationship with the onset age or the quality of dietary treatment (17). In the present study, 74.7% of the patients with abnormal EEGs had a low level of DQ. In addition, we found a significant relationship between EEG findings and the DQ level.

Ninety phenylketonuria (PKU) patients from one clinic were enrolled in a study performed by Rolle-Daya et al and the EEG findings were assessed. The patients were categorized in 3 groups. Group 1 included classical PKU patients who were diagnosed and treated early, manifested normal EEGs and made up 73% of the patients. In this group, 23% had mild background abnormalities, and 4% had paroxysmal discharges. Group 2 included patients in whom PKU was diagnosed after 6 months of age. In this group, only 31% had normal EEGs, 24% had background abnormalities and 45% had paroxysmal discharges. In group 3 who were patients with atypical PKU, 62% had normal EEGs and 38% showed background abnormalities. The start or end of the low phenylalanine diet caused no major changes in EEG patterns. No correlation was detected between the degree of dietary control, EEG findings, and intellectual performance in group 1 (18). In our study, a total of 63 patients (66.7%) had abnormal EEGs, 75 patients (79%) had a low level of DQ, and there was a significant relationship between EEG findings and the DQ level.

There is a report in which the results of two EEG studies on adult phenylketonuria (PKU) patients who were

treated early is addressed. In the first part of the study, the EEGs of 34 PKU patients were followed from birth to 21 years of age. The frequency of abnormal EEG findings (especially epileptiform activity) rose constantly up to the age of 12 but subsequently decreased. The quality of dietary control significantly correlated with IQ during the follow-up period. In the second part, frequency analysis of the EEG and neuropsychological testing were carried out on eight adult patients after periods of four weeks with low and high levels of phenylalanine. Only five patients followed the strict dietary regulations. High levels of phenylalanine caused a dominant peak of EEG background activity which shifted to the slower frequency spectrum in all patients. Neuropsychological testing showed cognitive function deterioration (19). Coskun et al conducted neurophysiological studies in 42 patients with classical phenylketonuria and intelligence quotient scores, electroencephalogram, visual evoked potentials and brain-stem auditory evoked potentials were evaluated. Normal intelligence quotient scores led to the conclusion that "evoked potentials may have a significant role in the determination of neurophysiological defects and that even cases with a good metabolic control may have some obscure neurophysiological dysfunction"; however, there is need for more careful evaluations (20). Distribution of DQ level in the case and control groups (abnormal and normal EEG patients) showed a significant difference; i.e. an abnormal EEG was associated with a higher percentage of low DQ levels (90.3% vs. 61.3%; p=0.001) (Table 5); therefore, based on our study, we believe that EEG findings may predict DQ scores in patients.

In conclusion, based on our findings, a significant difference was detected between the case and control group regarding DQ level. There was no significant difference in phenylalanine level between cases and controls; therefore, EEG findings may affect patients' developmental scores despite a normal phenylalanine level in PKU patients.

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