Clinical, Electroencephalographic (EEG), Neuroradiological and Molecular Correlations in Late-Detected Phenylketonuria (PKU) Patients

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ABSTRACT

The potential benefits of treating late diagnosed 60 patients with phenylketonuria (PKU) were investigated. Patients subjected to clinical, biochemical, IQ and electroencephalography (EEG) assessment and followed up in correlation with nutritional status. Further, a subset received magnetic resonance imaging (MRI). Screening for six common mutations (IVS10-11>G, R261Q, R252W, Y277D, E221D, V245V) was also performed. Patients were divided into different groups according to the onset of intervening year; imaging and molecular findings and the profiles of these groups were compared. Results showed that higher susceptibility to various patterns of seizures in 21 cases (35%) in the first two years of life however, this incidence decreased with age in spite of the elevated phenylalanine (Phe) level in blood. Alternately, EEG abnormalities increased with advancing age. Those exhibiting white matter abnormalities (WMAs) extending into subcortical/frontal regions (No=5) or WMAs with hypogenesis of corpus callosum (No=5) and or atrophy (No=11), displayed significant impairments in a number of domains. On the other hand patients showed no WMAs (No =10), or pathology restricted to the posterior periventricular region (No=15), displayed mild deficits. The most prevalent mutations were IVS-10-11 G>A (64.3%) and R261Q (35.7%). The 14 patients characterised (23.3%) were homozygous for the mutations that they carry. This is consistent with the high rate of consanguinity (71.1%) among families with PKU. Unexpectedly, hyperphenylalanemia and mild PKU have been detected in 4 of the patients` mothers. The data of the present study show that dietary restriction could substantially improve the most serious consequences of PKU even for late-diagnosed mentally retarded persons with PKU. (Egypt J. Neurol. Psychiat. Neurosurg., 2005, 42(2): 391-406).

INTRODUCTION

Phenylketonuria (PKU) is one of the first amino acid metabolic diseases to be characterized and the most common inborn error of amino acid metabolism in Caucasians, with an average incidence of 1/10,000¹. This autosomal recessive genetic disorder is caused by a deficiency of phenylalanine hydroxylase (PAH) enzyme. PAH is the rate-controlling enzyme of phenylalanine (Phe) homeostasis. In the liver, PAH, which requires tetrahydrobiopterin (BH4) as a cofactor, converts Phe, an essential amino acid, to tyrosine. Thus, Phe accumulates to plasma levels exceeding 1200 µmol/L and low plasma levels of tyrosine. The deficiency of PAH enzyme is caused by mutations in the PAH gene resulting in, intolerance to the dietary intake of Phe and production of the phenylketonuria (PKU) disease². The PAH gene, located at 12q22-q24.1, includes about 90kb and contains 13 exons. The degree of PAH enzyme impairment depends on the nature and position of mutations. To date,
more than 440 different alterations have been identified in the PAH gene. Deletions, insertions, point mutations, and splicing mutations have been described. In the Mediterranean region the most common mutation is IVS10-11G > A that is associated with severe phenotype. The number of possible mutations and the fact that most individuals are compound heterozygotes account only in part for the large biochemical and clinical phenotypic variability seen in PKU patients ranging from classical PKU to moderate hyperphenylalaninemia. It is noteworthy to mention that, impaired metabolism BH4 leads to malignant hyperphenylalanemia, which is more difficult to treat than PKU.

PAH deficiency is a highly heterogeneous trait that shows a broad spectrum of phenotypes. Plasma Phe levels of patients who are off-treatment may be above 1200 μmol/L or 20 mg/dl (‘classical PKU’), between 600 and 1200 μmol/L or 10-20 mg/dl (‘mild PKU’), or below 600 μmol/L or 2-10 mg/dl (‘non-PKU hyperphenylalaninemia’), as compared to levels of 40–120 μmol/L or < 2mg/dl in normal persons.

Timing is everything for children exposed to elevated levels of phenylalanine. The earliest possible recognition of disorders so that the early start of a phenylalanine-restricted diet can prevent the most serious consequences and the child’s development will be normal if the diet is adhered to. When dietary control is poor or late, PKU causes severe mental retardation, microcephaly, epilepsy, spasticity, tremor, clumsiness, and in some cases occasionally extrapyramidal features, owing to the damaging effects of hyperphenylalaninemia on the developing nervous system and effects related to insufficient tyrosine (fair colour of skin and hair and neuropsychological deficits). Seizures and imaging abnormalities in patients with PKU are consequences of hyperphenylalaninemia. However, It is a curious fact, that this association of PKU and seizures had scarcely been studied in the literature, although there have been numerous short notes or case reports in literatures. Interestingly, white matter abnormalities (WMAs) found in PKU patients either early treated or late treated. The WMAs are thought to represent elevated water content in the myelin and, possibly, disturbed myelin synthesis. They are found predominantly in the posterior periventricular cerebral white matter, but in more severe cases they also extend into anterior and subcortical regions. Hypoplasia of the corpus callosum is a feature of maternal PKU and is probably a result of inhibition of corpus callosum development at 8 to 20 weeks of gestation. Moreover, cerebral atrophy could be the late result of chronic exposure to high phenylalanine concentrations. The incidence and severity of WMAs increase with age, but it seems to be individual variation in the vulnerability of the brain to blood phenylalanine levels. The clinical significance of PKU-related WMAs is not known, although there is some evidence to suggest a possible link between PKU-related WMAs and cognitive impairment especially WMAs pathology extending into subcortical and/or frontal regions are at increased risk for significant neuropsychological deficits. A raised Phe level may impair transport into the brain of other large neutral amino acids that share the same transporter, including tyrosine and tryptophan, with resultant alteration in neurotransmitter levels. Consequently, the neurons of the dopamine-dependent prefrontal cortex have specific characteristics, causing this area of the brain to be particularly sensitive to fluctuations in dopamine precursor tyrosine and this could be the cause of neuropsychological deficits found mainly in speed of information processing and higher integrative functioning and attention deficit hyperactivity disorder (ADHD)-inattentive type.

The present study thought to determine seizure, imaging abnormalities and EEG abnormalities frequency and type in a cohort of males and females with late treated PKU according to the intervening year, to examine further the relationship between WMAs and its impact on the IQ, and seizure frequency (children were differentiated according to the severity of their pathology). More interestingly to present the
pregnancies outcomes of untreated females with maternal hyperphenylalanemia and mild PKU and to present simple correlation between the genotype of two mutations and the clinical phenotype.

**PATIENTS AND METHODS**

Sixty cases with typical PKU (serum Phe level more than 20 mg/dl.) were evaluated. These patients were diagnosed in our Clinical Genetics Department from July 1997 through January 2005. Severe learning disability, poor behavior control, hyperactivity, seizures and delayed speech development were common presenting complaints. Moreover, neurological deterioration and seizures was the complaint of two cases. A detailed medical history including family history, consanguinity, similarly affected family members (pedigree), case history of mothers and fathers, history of the present condition (date of diagnosis, onset of initiation of low Phe diet) and seizure type and frequency were obtained for each patient.

Patients were divided into four groups according to the onset of intervening year: group 1 (started after the age of 6 month but at 1st year of life; n = 9), group 2 (started at 2nd year of life; n = 17), group 3 (started from 3 to 6-year-old; n = 27) and group 4 (started after the age of 6; n=7). All the 60 cases were subjected to anthropometric examination (weight, height and head circumference) and full clinical evaluation with special emphasis on the neurological assessment regarding tone, reflexes, Babinski sign, gross motor function, function of the lower extremities and fine motor functions.

**Biochemical:** Blood Phe concentrations for all the patients were determined by the enzymatic colourimetric method in dried blood spot (Quantase). Typical PKU was diagnosed according to serum phenylalanine (Phe) level more than 20 mg/dl. In addition, Phe concentration were evaluated for 20 mothers who had more than one affected child with PKU, congenital microcephaly and or congenital heart disease with PKU.

**Diet:** Current treatment of PKU consists of a Phe-restricted diet (well-adjusted to the tolerance) supplemented with a tyrosine-, vitamin-, and oligoelement-enriched amino acid mixture or with a specific formulation high in all the other amino acids necessary for protein synthesis.

**EEG** was performed for all patients using the international 10-20 system of electrode placement with sedation (by chloral hydrate). The length of the EEG recording was 20 minutes with hyperventilation in co-operative cases but without photic stimulation. Further, the previous EEGs were also reviewed. For each case with epilepsy, extensive data collection through personal interview was done by two of the authors concerning the onset, type, frequency, and response to treatment. We reviewed 137 EEGs from 60 patients with PKU. Moreover, EEG was performed to the 4 mothers who showed elevated Phe level.

**Intelligence quotient and/or Developmental Quotients (IQ/DQ):** All the patients were tested using Wechseler test and/or portage scale for evaluating the developmental quotient in young patients (up to 5–year-old). The degree of mental retardation (MR) was evaluated according to WHO classification: normal >80, borderline =70-79, mild = 51-69, moderate = 36-50, severe = 21-35, profound = 0-20.

**CT and/or MRI:** These examinations were performed for 46 patients (76.7%). Mild white matter abnormalities (WMAs) if confined to the posterior periventricular region, moderate if extending into subcortical and/or frontal regions and severe if associated with hypogenesis of corpus callosum. Rating scales were used to grade the severity of imaging abnormalities: 0 = normal 1 = mild WMAs, 2 = moderate WMAs, 3 = severe WMAs, 4 = if WMAs associated with brain atrophy.

**Molecular Study of the PAH Gene**

Genomic DNA was isolated from peripheral blood samples using the salting out procedures as described by Miller et al. Cases were screened for six mutations (IVS-10-11 G>A, R261Q,
R252W, Y277D, E221D and V245V) by polymerase chain reaction (PCR) of the PAH gene followed by restriction analysis. This entailed amplification of the mutation site and its flanking sequences using specific primers followed by restriction enzyme cutting within the mutation sites following the procedure of Eiken et al.23

Statistical analysis of data, Package for Social Science (SPSS for Windows Release 6; SPSS Inc., Chicago, IL, USA) was used. Simple linear regression (rs) was used to evaluate the relationship between epilepsy and the degree of MR, head circumference, height, body weight of patients at time of assessment. In addition, to evaluate the relationship between the grades of MRI abnormalities and the degree of MR, head circumference, height, and body weight of patients at time of assessment. Student t-test was used to compare the means of continuous variables between different groups. For the evaluation of categorical variables, Chi square test was used. All tests were two sided and P values < 0.05 were considered significant.

RESULTS

General features
This study included sixty cases from 45 families with classic PKU. Age range of the children was from 6 months to 22 years with the mean of 5.65±0.67 year. Sex ratio was 0.51 in the total group. Mean maternal age at the birth of affected child was 27 ±0.92 years and the mean paternal age was 32±0.91 years. Parents having 1 child with PKU or 2 and 3 children with PKU represented 60.8% 28.3% and 10.9%, respectively, of families surveyed. Four of these mothers proved to have high Phe level (3 with hyperphenylalanemia and one with classic PKU). Mean Z-scores for weight, height and head circumference of the PKU children were -0.53 SD, -0.92 SD and -1.67 SD, respectively. Moreover, 20 cases (33.3%) had microcephaly (head circumference <2 SD). All mothers who showed high Phe level had microcephalic PKU patients but none of them had congenital heart disease. Three cases (5%) with PKU had associated congenital anomalies in the form of spina bifida occulta, mitral prolapse and albinoid fundus. In addition to a unique occurrence of PKU in a patient with Down syndrome (Fig. 1). An important finding, consanguinity was documented in 32 families (71.1%) and our sample included 12 sibships 9 of them from consanguineous marriage. Calculated sibling recurrence risk for all the cases was 32.8 % with mean inbreeding coefficient 0.0630.

Seizures and intelligence
Of the 60 cases with classic PKU, (Table 1) 21 cases (35%) had epilepsy. In this study, there was higher prevalence of epilepsy in males 66.7% versus 33.3% in females. Twenty cases had the age of onset of epilepsy at the first year of life (95.2%). The main seizure type was generalized tonic-clonic seizures which was evident in 11 cases (52.4%), however, 3 patients (14.3%) had west syndrome and 7 (33.3%) cases with focal or partial seizures. Sixteen cases (76.2%) were well controlled 2 of them were well controlled only on diet while the rest (14 cases) on diet and antiepileptic drugs. On the other hand 5 (23.8%) had partially controlled seizures.

The IQ of the PKU patients ranged from mild to profound mental retardation. The mean IQ for our 60 patients in the study was 47.7±2.62.

An important finding was the inverse correlation between the occurrence of epilepsy and the IQ (rs = -2.5, p=0.01). Meanwhile, no correlation was observed between the occurrence of epilepsy and head circumference at time of assessment of all patients in the study (rs = -0.16, p=0.3). The body weight, height and biochemical phenotype of all patients at time of assessment also inversely correlated with epilepsy (rs = -0.08, -0.12, -0.2 and -0.961, respectively) however, they did not reach the level of statistical significance.

As shown in table (1), the number of cases is limited in different subgroups for a precision statistical analysis. Group 1 and 2 had higher
incidence of epilepsy when compared with group 3 and 4, however, the incidence of brain imaging abnormalities did not show marked difference between groups (Table 1). In our group of patients there was minimal effect on the IQ of the timing of diet onset.

**EEG changes**

PKU patients who had no history of epilepsy at any stage of their life showed normal EEGs in 69.2% (27/39) of the cases. Meanwhile, focal paroxysmal discharge (Fig. 2) and subcortical epileptogenic dysfunction (fig.3) were seen in 7 (58.3%) and 5 (41.7%) cases respectively. Three of these cases (3/12; 25%) had normal EEGs in infancy but abnormal EEGs when retested later even 2 of them though remain on a relaxed diet. On the other hand, severe alterations were seen in epileptic patients. The EEG of patients with epilepsy (n=21), showed focal spikes, generalized spikes/waves or mixed. In addition, hypsarrhythmia (fig. 4) was recognised in 3 patients (14.3%). Group classification showed higher incidence of EEG abnormalities among cases in group 3 and 4 compared with group 1 and 2. EEG done for the four mothers who showed high level of Phe revealed normal pattern. In epileptic patients with infantile spasm, there was significant association between the initial EEG pattern and response to diet and antiepileptic treatment and between clinical remission and EEG normalization and long-term seizure control.

**MRI findings**

The overall incidence of imaging abnormalities was 78.3% (36/46 cases). Fifteen patients had pathology restricted to the posterior periventricular region (mild WMAs) and 5 patients (Fig. 5) had pathology extending into subcortical and/or frontal regions (moderate WMAs). White matter abnormalities and thining of corpus callosum (Fig. 6) were evident in five cases (severe WMAs). Moreover, one of them had cerebellar atrophy (Fig. 7). Further 11 cases showed WMAs and brain atrophy (Fig. 8) and the remaining 10 patients had no detectable abnormalities (Table 1).

An important finding was the significant inverse correlation between the severity of MRI findings and the IQ (rs=-2.962, P<0.005). In the meantime, there was significant correlation between the severity of abnormal MRI findings and occurrence of seizures (rs=3.25; P<0.002). In addition, no correlation was observed between the severity of abnormal MRI findings and age or biochemical level (p=0.06 and p=0.48, respectively). These data indicate an association between severity of MRI findings and occurrence of seizures and the low IQ values among cases with PKU.

Next step in our data analyses involved grouping of the cases according to the type of brain abnormalities and comparing them with cases with no detectable brain abnormalities (Table 2). Cases with mild, moderate and severe brain abnormalities showed decrease in the IQ values when compared with cases with no detectable brain abnormalities, although it was significant in the groups that showed severe white matter abnormalities and group with brain atrophy. Further, group with moderate and severe white matter abnormalities showed significant decrease in the head circumference but increase in the incidence of seizures.

**Therapy and prognosis**

All PKU patients were treated with low phe diet immediately after the diagnosis of PKU was established. It was a major challenge because most of these cases who never have been exposed to the relatively unpalatable Phe-free medical product essential for metabolic control. With the start of diet two patients (9.5%) who received only diet therapy were seizures free without any antiepileptic drugs (AEDs). On the other hand, 14 (66.7%) patients who received a combination of low phe diet therapy and AEDs (valproate and/or nitrAZepam) ceased to show seizures within 9 months after initiating the therapy and only two patients relapsed. While, 5 patients (23.8%) showed partial control.

In group 1, 2 and 3, the hyperactivity and autistic behavior was gradually decreased when
serum Phe concentrations dropped to 6 mg/dl or below even 11 patients (20.7%) showed intellectual improvement after diet therapy. In group 4, patients failed to adhere to diet. Hyperactivity, aggression and autistic behavior were marked but showed some improvement on valporates and risperidone.

Biochemical phenotype:
The mean Phe level of the patients ranged from 20 to 36 mg/dl with the mean of 24.8±1.3 mg/dl. The mean of Phe level among groups is presented in Table 1. An important finding was high Phe level found in 4 of the PKU mothers (4/20; 20%). One of them had mild PKU (15 mg/dl) while the others had hyperphenylalanemia (6 mg/dl). This could partially explain the high sibling recurrence risk.

Mutation screening in correlation to clinical and biochemical phenotype.
Screening for six mutations (IVS10-11>A, R261Q, R252W, Y277D, E221D, V245V) that are relatively common in this geographical area allowed the characterization of 23.3% (14 cases) of cases. 64.3% of the mutant alleles are IVS10-11>A (9 cases). Only 35.7% alleles were R261Q (5 cases). However, other mutations were (R252W, Y277D, E221D, V245V) not detected. The biochemical phenotype of the 14 patients with the two mutations did not differ significantly (Table 2). It was noted that 19.04% of epileptic patients (4 cases) were harboring IVS10-11>A mutation in contrast to 4.8% (one case) that showed R261Q. None of these cases had west syndrome. Of 14 patients with homozygous IVS10-11>A alleles and R261Q mutations, 19.4% (7/36) versus 5.5% (2/36) exhibited brain imaging abnormalities, respectively. In the former group, one patient showed partially controlled seizures. The biochemical phenotype of the 14 patients with the two mutations did not differ significantly (Table 2). It was noted that 19.04% of epileptic patients (4 cases) were harboring IVS10-11>A mutation in contrast to 4.8% (one case) that showed R261Q. None of these cases had west syndrome. Of 14 patients with homozygous IVS10-11>A alleles and R261Q mutations, 19.4% (7/36) versus 5.5% (2/36) exhibited brain imaging abnormalities, respectively. In the former group, one patient showed partially controlled seizures. The mean IQ of IVS10-11>A patients was significantly lower than those who had R261Q (41.9± 5.5 vs 61 ± 5.8) p =0.02. However, one patient with IVS10-11>A (1/11; 9.1%) showed intellectual improvement after diet therapy. Alternately, one of the patients with R261Q did not show any intellectual improvement although diet intervening was earlier than the IVS10-11>A patient.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of patients with PKU according to the subgroup classification.</th>
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<tbody>
<tr>
<td><strong>Characteristic</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Sex ratio (M/F)</strong></td>
</tr>
<tr>
<td><strong>Phenylalanine level (mg/dl)</strong></td>
</tr>
<tr>
<td><strong>IQ</strong></td>
</tr>
<tr>
<td><strong>Weight (SD)</strong></td>
</tr>
<tr>
<td><strong>Height (SD)</strong></td>
</tr>
<tr>
<td><strong>Epilepsy (%)</strong></td>
</tr>
<tr>
<td><strong>Abnormal EEG (%)</strong></td>
</tr>
<tr>
<td><strong>MRI abnormalities (%)</strong>****</td>
</tr>
<tr>
<td><strong>White matter abnormalities (No)</strong></td>
</tr>
<tr>
<td><strong>Atrophic changes (No)</strong></td>
</tr>
<tr>
<td><strong>No. of cases with IVS10-A G</strong></td>
</tr>
<tr>
<td><strong>No. of cases with R261Q</strong></td>
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</tbody>
</table>

* Incidence of epilepsy was significant statistically when compared with group 1 and 2 (P= 0.048 and 0.029, respectively)
Table 2. Characteristics of PKU patients with brain imaging abnormalities compared with those having no brain imaging abnormalities.

<table>
<thead>
<tr>
<th></th>
<th>Patients with no brain abnormalities (No= 10)</th>
<th>Patients with mild WMAs (No= 15)</th>
<th>Patients with moderate WMAs (No= 5)</th>
<th>Patients with severe WMAs (No= 5)</th>
<th>Patients with brain atrophy (No= 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.7</td>
<td>5.2</td>
<td>2.2</td>
<td>4.04</td>
<td>7</td>
</tr>
<tr>
<td>Weight (SD)</td>
<td>0.4</td>
<td>-0.6</td>
<td>-0.8</td>
<td>-0.2</td>
<td>-0.5</td>
</tr>
<tr>
<td>Height (SD)</td>
<td>0.1</td>
<td>-1.1*</td>
<td>-1.4*</td>
<td>-1.4*</td>
<td>-0.9*</td>
</tr>
<tr>
<td>Head circumference (SD)</td>
<td>-1.1</td>
<td>-1.6</td>
<td>-2.4*</td>
<td>-2.2*</td>
<td>-1.01</td>
</tr>
<tr>
<td>Phe level (mg/dl)</td>
<td>23.8</td>
<td>24.3</td>
<td>26.7</td>
<td>25.7</td>
<td>26</td>
</tr>
<tr>
<td>IQ</td>
<td>60</td>
<td>48.9</td>
<td>46.6</td>
<td>34*</td>
<td>33.6*</td>
</tr>
<tr>
<td>Seizures (no; %)</td>
<td>-</td>
<td>6 (40%)</td>
<td>4 (80%)*</td>
<td>4 (80%)*</td>
<td>5 (45%)</td>
</tr>
</tbody>
</table>

Patients with no brain abnormalities were taken as referent in the statistical analysis.

* P< 0.05 ** P< 0.005

Table 3. Characteristics of patients with PKU according to the mutation type.

<table>
<thead>
<tr>
<th></th>
<th>IVS10-11&gt;A (No= 9)</th>
<th>R261Q (No= 5)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.3</td>
<td>12</td>
<td>0.12</td>
</tr>
<tr>
<td>Sex ratio (M/M+F)</td>
<td>0.55</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Epilepsy (%)</td>
<td>4 (19.04%)</td>
<td>1 (4.8%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Abnormal EEG (%)</td>
<td>2 (16.7%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MRI abnormalities (%)</td>
<td>8 (22.2%)</td>
<td>2 (5.6%)</td>
<td>1</td>
</tr>
<tr>
<td>Phenylalanine level (mg/dl)</td>
<td>25.6±1.7</td>
<td>23±0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>IQ</td>
<td>41.9±5.5</td>
<td>61±5.8</td>
<td>0.02*</td>
</tr>
<tr>
<td>Weight (SD)</td>
<td>-0.53±1.4</td>
<td>-0.2±0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Height (SD)</td>
<td>-0.98±0.3</td>
<td>-0.12±0.4</td>
<td>0.09</td>
</tr>
<tr>
<td>Head circumference (SD)</td>
<td>-1.9±0.43</td>
<td>-0.4±0.5</td>
<td>0.04*</td>
</tr>
<tr>
<td>Mild WMAs (No. of cases)</td>
<td>6</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Moderate WMAs (No. of cases)</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Severe WMAs (No. of cases)</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Atrophic changes (No. of cases)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

Fig. (1): Facial picture of Egyptian patient with classic PKU and the unique association with Down syndrome.
**Fig. (2):** EEG in classic PKU girl aged 3-year old showing frequent focal sharp waves over either fronto-temporal regions. Background activity of 3-6C/s delta waves and sleep spindles.

**Fig. (3):** EEG in classic 1.5-year old boy with PKU showing 3-6 C/sec delta and theta waves mixed with sleep spindles. There were recurrent periodic paroxysms of sharp and notched slow waves suggesting subcortical epileptogenic dysfunction
Fig. (4): EEG in classic 6-month-old girl with hypsarrhythmia.

Fig. (5): Flair axial T2 MRI showing abnormal high signal intensity in the deep white matter region around anterior and posterior horns and the body of both lateral ventricles (grade 2).
Fig. (6): (A) axial T2 weighted MRI showing abnormal high signal intensity in the deep white matter region around anterior and posterior horns and the body of both lateral ventricles  (B) Mid sagittal MRI view of brain; note thinning of corpus callosum (grade 3).

Fig. (7): A) axial T2 weighted MRI showing abnormal high signal intensity in the deep white matter region around the body and posterior horns of lateral ventricles (B) Coronal MRI showing high signal intensity in the deep white matter region around anterior horns of both lateral ventricles  (c) Mid sagittal MRI view of brain; note agenesis of corpus callosum and vermal atrophy (grade 3).

Fig. (8): A) axial T1 weighted MRI showing abnormal high signal intensity in the deep white matter region around anterior and posterior horns and the body of both lateral ventricles and brain atrophy (B) Mid sagittal MRI view of brain; note agenesis of corpus callosum (grade 4).
In this study we investigated the impact of late diagnosed PKU patients on the occurrence of epilepsy, EEG changes and brain imaging abnormalities that might be sensitive to long-term elevation of phenylalanine levels and correlating these findings to the mutations screened.

The incidence of epilepsy observed in this study was 35% on 60 Egyptian patients with PKU compared to 25.5% recorded in 603 Chinese patients with PKU. This apparent slightly higher incidence may be attributed to the number of the tested samples. It is rather difficult to classify epilepsy (secondary to metabolic disorders) into precise epileptic syndromes. However, the association of west syndrome with PKU has long been recognised in literatures since Low et al.’s first report in 1957. Similar frequency of west syndrome was 14.3% among our cases in contrast to 12.3% reported by Zhongshu et al.

By differentiating our 60 cases into four groups according to the intervening year, (despite the variation among individuals within these groups) certain conclusions are apparent. PKU patients showed higher incidence of epilepsy in the first year of life and this finding is consistent with the literature. In addition, this incidence decreases with age in spite of the elevated Phe level in blood. On the other hand, older non-epileptic patients (in group 3 and 4) were more likely to have abnormal EEGs. In another word no one of the non epileptic patients in group 1 and 2 had abnormal EEGs before the age of 3 had abnormal records. This observation had also been reported in prospective studies of PKU. So it is most likely that EEG abnormalities increased with advancing age independently of MRI abnormalities and showed no relation to IQ development.

Given the ongoing debate about the clinical importance of WMAs and whether it affects the cognitive function, we were particularly surprised that our sample of late-detected PKU patients showed lower incidence (78.3%) of white matter abnormalities (WMAs) than that reported in early treated PKU samples. However, the age distribution of our sample was significantly younger. It is noteworthy to mention that Anderson et al. reported on similar incidence of WMAs (81.3%) in his sample and regarded that to the same reason. So, it seems likely that the incidence of WMAs increase with advancing age. Taking into consideration that the regional distribution of the WMAs does not seem to be age dependent as infants and adults show the same regional distribution pattern or related to the intervening time as it is found in early, late, and untreated patients. Children without detectable WMAs also displayed deficits but better in the mean of IQ, head circumference values and incidence of seizures when compared with cases with brain abnormalities. Further, our results showed that the severity of brain abnormality (the extent of MRI abnormalities) was most strongly associated with low IQ values but not to the blood Phe concentration at the time of imaging. This finding strongly supports hypothesis of Anderson et al. In addition, not only WMAs, that extend beyond the posterior periventricular region but also hypoplasia of corpus callosum may adversely affect IQ.

In view of the results that no relationship had been found between occurrence of epilepsy and severity of brain lesion and level of serum Phe, it is interesting to confirm the same results obtained from recent study by Brumm et al. Nevertheless, this is not a universal finding as many previous studies showed significant relationship to serum Phe level.

Confirming results from previous study that the most common mutations in Egypt are the IVS10-11G>A and R261Q and comparing our results with reported mutations. These mutations include R408W in China, Eastern Europe, East Russia, Germany and Brazil, R413P in Japan, IVS10-11G>A in the Mediterranean and Iran, IVS12→1G>A in Denmark and England, Y414C in Scandinavia, I65T in Western Europe. In addition, R261Q is common mutation in Brazil, Iran, Sicily, France, Netherlands, Cuba and South
Italy. Moreover, R252 was reported as a common mutation in Italy, Iran, Chile, Brazil, Portugal\(^4,36-40\).

After the identification of mutations in 23.3% of our patients the interest lies in the elucidation of the clinical phenotype underlying these mutations. IVS10-11G>A mutation correspond to the category of second most frequently reported in all populations especially Mediterranean populations\(^5\). Nine cases (15%) were homozygous for this mutation. Further, 5 cases (8.3%) were homozygous for R261Q mutation. Generally, PKU has been cited as an example of conformational disease in which the increase in degradation of folding intermediates and improperly folded proteins is the main molecular mechanism. IVS10-11G>A mutation had a putative folding defect that showed no residual effect of the PHE and that explains that it is considered as severe mutation. On the other hand R261Q are folding defects as well but it is associated with low-intermediate levels of activity (10–70%)\(^6\). R261Q causes reduced stability and accelerated degradation.

Based on the results obtained in this work, patients with IVS10-11G>A showed significant lower IQ values, smaller head circumference higher incidence of imaging abnormalities and epilepsy with bad response to AEDs and diet control when compared with cases with R261Q. In view of these results obtained, it is interesting to confirm that the clinical impact of the mutations corresponds to the severity estimated by Pey et al\(^3\). Although this observation is interesting, but it shouldn’t be taken as a hard evidence because the number of children in this sample was small. In addition, late treated PKU patients do not have IQ scores fully concordant with the predicted severity of the PAH genotype\(^31\) and close correlation between its metrical value in PKU subjects and the PAH genotype requires cautious interpretation and needs further research. It is noteworthy to mention, it was suggested that R408W mutation that cause no residual effect of the PHE (similar to IVS10-11G>A) predispose to mental illness\(^32,33\). One of the major questions remaining in PKU research is why cases who share the same mutations IVS10-11>A in the phenylalanine hydroxylase (PAH) gene had different disease courses as regard to occurrence of seizures or not, type of seizures and response to dietary treatment and outcome. We suppose that in IVS10-11>A mutation, the differences in the courses of the disease between cases appear to be related to variations in their blood-brain barriers and suggesting that there is not a simple correlation between genotype and intellectual phenotype\(^42\). Alternately, variation in cases with R261Q mutation could be regarded to individual variation in the quality control system\(^3\).

Hoping for complete characterization of PKU mutations in Egypt so, reasonable predictions of the phenotype of a large number of patients may be deduced. The homozygosity for the mutation is due to the high rate of consanguineous marriages in the family (71.1%) of the patients. Although this was a preliminary study on the analysis of PKU in Egypt, but the data obtained could be used as a base for further investigation of the disease in this population.

Another interesting observation was that 39.2% of the mothers have more than one affected child with PKU which attracted our attention to determine the blood Phe level for these mothers and to our surprise that 3 of them had non-PKU mild hyperphenylalaninemia and another one had mild PKU. A particular question is whether maternal non-PKU mild hyperphenylalaninemia (MHP) presented a threat to the fetus. Retrospective international survey of untreated maternal MHP concluded that this entity did not have serious consequences for the fetus, although the birth measurements and IQ scores were slightly lower in offspring when maternal blood Phe was > 400 µmol/l and these authors found that at least 30% of those infants were identified to have hyperphenylalaninemia\(^35\). Unexpectedly, our series comprised six children with classic PKU (rather than hyperphenylalaninemia) born to 4 mothers of them with HPA and the other with classic PKU. This could be regarded to the additive deleterious effect of consanguinity. This finding shows that although the inheritance of the mutant genotype is recessive, PKU is a complex trait.
Confirming the impact of high blood Phe on brain growth, all these sibs had congenital microcephaly, white matter abnormalities and hypogenesis of corpus callosum and low IQ values but none of them had congenital heart disease. Nevertheless, hypoplastic corpus callosum could be a marker for brain effect in maternal hyperphenylalaninemia. Although there is no established precise relationship between maternal phe levels and outcome\textsuperscript{46}. Women with HPA need early identification, education regarding the importance of dietary compliance, careful monitoring, and emotional support to increase the chance for a better outcome.

Given heterozygote frequency of 1:50 to 1:70\textsuperscript{47}, several mechanisms have been proposed to explain the relatively high PKU frequency in humans including founder effect/genetic drift, selective advantage of heterozygotes, reproductive compensation, high mutational rate, and involvement of multiple loci that give rise to similar disease phenotypes\textsuperscript{48}. Down syndrome (Trisomy 21) is the most prevalent unbalanced chromosomal aberration with an incidence of 1:600 live birth or 1: 150 conception\textsuperscript{49}. Our series comprised another unique association of PKU and Down syndrome. The association of these two rare conditions could be due to random association of the two syndromes. It is noteworthy to mention that PKU has been reported in association with Goldenhar's syndrome\textsuperscript{50}.

In conclusion, the high incidence of seizure in first two years of life is due to high Phe level whereas it is well controlled on diet and AEDs. While the abnormal EEG and MRI changes are secondary to the chronic exposure to high Phe. Although, Phe levels were not correlated with severity of the MRI abnormalities, incidence of seizures or IQ values but indeed lifetime Phe were associated with deficits in several aspects. Accordingly, seizures, EEG and /or MRI and neurological manifestation are at least partially reversible by lowering the blood Phe concentration at any age especially before the age of 6 year old. Based on this results patients with IVS10-11G>A showed significant lower IQ values, smaller head circumference higher incidence of imaging abnormalities and seizure with bad response to AEDs and diet control when compared with cases with R261Q.

It is very important to carry out neonatal screening program in Egypt for prevention of PKU and reducing the burden of disease. Laboratory testing is only availability of testing to all individuals. Education regarding the program, effectiveness of low Phe diet, long-term benefits both for individuals and society, ethical issues, and cost benefits has to be cleared.

**REFERENCES**


الملخص العربي

الظواهر الإكلينيكية، رسم المخ الكهروسكوبي، التصوير التษيلي، وعلاقتها بمزضى الفينيل كيتوينوريا الذين بدأوا العلاج متأخراً

ينتولد البحث مدى الجدوى واستفادة الأطفال المصابين بمرضى الفينيل كيتوينوريا الذين بدأوا العلاج متأخراً من النظام العصبي الحالي من الفينيل الآثدين. أثمينة الدراسة على الفحص الإكلينيكى، التقييم الروتيني، والتشخيص البيولوجي لمسبة الفينيل الآثدين، بالإضافة إلى تقييم حالات الفحص بالرنين المغناطيسي وكذلك تحديد الطفرة الوراثية لبعض الحالات. فلقد قمنا بتقسيم الحالات إلى مجموعات بناءً على العمر الزمني الذي بدأ فيه الإتزام بالمرضى الجاذب من الفينيل الآثدين، 1. التغييرات التي وجدت في الرنين المغناطيسي با لع واعحاً بناءً على نوع الطفرات الوراثية وعمل مقاربات إحصائية بينها. أظهرت النتائج أن معدل حدوث التغييرات ذات الالوان المختلفة كان بنسبة 35% في 21 حالة وذلك في السنة الأولى من العمر هذا وقد قمنا نسبي حفظ التغييرات مع نمو العمر.

و على العكس فقد زادت تغييرات رسم المخ مع نمو العمر على الرغم من أن نسبة الفينيل الآثدين بالدم كانت عالية في الحالات. وقد أظهرت الدراسات الإحصائية أن تغييرات الماذية البيضاء والتي أظهرها الينين المغناطيسي والتي أظهرت الالي لفاص الامامي للدم والنرجس النطرة تلعب دوراً هاماً في ازدياد نسبة التغييرات وانخفاض مستوى النزاع، وظهور تغييرات رسم المخ. وعلى العكس في الحالات التي اقترضت تغيرات الماذية البيضاء فيهما على الجانب القلبي لتجولي المخ أو الحالات التي لم يظهر الینين المغناطيسي للدم فيها أي تغييرات.

ظهرت الطفرات الوراثية المتماثلة في 23.3% من الحالات وتمثل الطفرة المتماثلة في 64.3% IVS-10-11 G>A من الحالات بينما ظهرت R261Q في 35.7% من الحالات. ادي زواج الأقارب (في 71.1% من الحالات) الى ظهور هذه الطفرات المتماثلة. وعلى غير المتوقع ظهير التحليل الوراثي البيولوجي في نسبة الفينيل الآثدين بالدم عالية لدى أربعة من أمهات الأطفال المصابين بمرضى الفينيل كيتوينوريا.

تؤكد هذه الدراسة على أهمية الالتزام بالأطفال المصابين بمرضى الفينيل كيتوينوريا بالنظام الغذائي الدقيق من الفينيل الآثدين حتى لو بدأ في سن متاخرة ربما في ذلك من نقلات الحفظ التشيكل لدى اختصار العطر في مسبوكي النتزاع والتغييرات بالمادة البيضاء بالمغ.