

## Incidence of Neonatal Hyperphenylalaninemia in Fars Province, Southern Iran

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### Abstract

**Objective:** Phenylalanine hydroxylase or its cofactor, tetrahydrobiopterin (BH<sub>4</sub>), deficiency causes accumulation of phenylalanine in body fluids and central nervous system. Considering the fact that hyperphenylalaninemia is a preventable cause of mental retardation in infants, the objective of this study was to determine the incidence of congenital hyperphenylalaninemia in Fars province, south of Iran.

**Methods:** In a period of one year from November 2007 to November 2008 blood samples were withdrawn from all newborns born in Fars province for measurement of serum phenylalanine. The samples with a serum level of  $\geq 2$  mg/dl were referred to pediatric endocrine clinic for confirmation and determination of the type of hyperphenylalaninemia by quantitative serum phenylalanine measurements by using High-Pressure liquid chromatography (HPLC) method.

**Findings:** Nine out of 76966 newborns had a serum phenylalanine level  $\geq 2$ mg/dl, of which 8 cases were confirmed by HPLC. The incidence of the disease was 1:10000. The incidence of mild hyperphenylalaninemia and phenylketonuria (PKU) among the patients was 62.5% and 37.5% respectively and the incidence of BH<sub>4</sub> deficiency was 1/76966.

**Conclusion:** These findings indicate a high incidence of hyperphenylalaninemia, in the newborns from Fars province. The high incidence makes a comprehensive screening program for management of the disease necessary.

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**Key Words:** Hyperphenylalaninemia; PKU; BH<sub>4</sub>; Screening; Newborn

### Introduction

Phenylalanine is an essential amino acid. The enzyme phenylalanine hydroxylase and its

cofactor tetrahydrobiopterin catalyze the conversion of phenylalanine to tyrosine, and their deficiency results in accumulation of phenylalanine in body fluids and central nervous

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system<sup>[1,2,3]</sup>. The severity of hyperphenylalaninemia depends on the degree of enzyme deficiency and may vary from highly elevated concentrations (more than 20 mg/dl) or classic phenylketonuria (PKU) to mildly elevated levels (2-6 mg/dl)<sup>[3]</sup>. Affected infants are normal at birth, but if the diagnosis is delayed and phenylalanine-restricted diet is not considered for the affected infant, the signs of mental retardation will gradually appear. Considering the fact that hyperphenylalaninemia is a preventable cause of mental retardation, screening in the first days of birth and rapid diagnosis is of utmost importance. The incidence of hyperphenylalaninemia in different countries is variable. To the best of our knowledge there are no reports on the incidence of PKU in Iran. This study is designed to estimate the annual incidence of congenital hyperphenylalaninemia in Fars province (Iran) during the period of November 2007 to November 2008 and also to investigate different types of hyperphenylalaninemia.

## **Subjects and Methods**

In this cross sectional study the status of screening programs for congenital hyperphenylalaninemia was assessed and their results in Fars province in the period of November 2007 to November 2008 were studied. All infants in Fars province who were born between November 2007 and November 2008 were included in this study. Because birth certificate is issued only after the completion of screening tests, so one can be sure that all newborns born during this period are included in the study. Neonates underwent screening study for hyperphenylalaninemia using Guthrie method between their 3<sup>rd</sup> and 5<sup>th</sup> day of life.

Those who had phenylalanine levels above 2 mg/dl in screening test were considered positive and were referred to endocrinology clinic (Shiraz University of Medical Sciences) for further evaluation.

After taking complete history and physical examination (height, weight, and head circumference measurement) their serum and

urine phenylalanine levels were determined. Measurement of phenylketons in the urine has no place in any screening program but its presence in the urine simply signifies the severity of the condition<sup>[3]</sup>. High Pressure Liquid Chromatography was applied for the determination of serum phenylalanine-tyrosine levels. A serum value above 2 mg/dl accompanied with normal tyrosine level was labeled as definite hyperphenylalaninemia.

Depending on the serum level of phenylalanine the affected infants were divided in two groups: classic hyperphenylalaninemia (>20 mg/dl) and mild hyperphenylalaninemia (2-6 mg/dl). All the patients were followed by a monthly serum phenylalanine level using High-Pressure liquid chromatography (HPLC) method.

For the second phase of the study tetrahydrobiopterin (BH<sub>4</sub>) deficiency was performed by the BH<sub>4</sub> loading test in order to determine which of the affected infants had BH<sub>4</sub> deficiency and would respond to BH<sub>4</sub> therapy<sup>[3,4]</sup>. First, the patient's phenylalanine-restricted diet was discontinued for two days and the infant had a normal diet to let the serum phenylalanine level rise. On the morning of the third day the basic serum phenylalanine level was checked using HPLC method. After that, BH<sub>4</sub> tablets were administered (20 mg/kg) and serum phenylalanine levels checked at 4 hours, 8 hours and 24 hours thereafter<sup>[3-7]</sup>. Normal serum phenylalanine in any of the three measurements was considered as response to BH<sub>4</sub> and the patient was accepted as BH<sub>4</sub> deficient. The percentage of drop in serum phenylalanine level was also calculated.

## **Findings**

During the one year of study, a total of 76966 live infants were born in Fars province, of whom 35470 (46%) were girls and 41496 (54%) were boys. In screening tests 9 cases were found to have increased levels of phenylalanine and were referred to the endocrine clinic. One subject had serum phenylalanine below 2 mg/dl after two tests using HPLC method and was excluded from the study. The other 8 infants had serum

phenylalanine levels above 2 mg/dl. Three out of 8 patients had serum phenylalanine levels above 20 mg/dl and urine was positive for phenylketone (classic PKU). Five infants had serum phenylalanine levels between 2 and 20 mg/dl (mild hyperphenylalaninemia). All patients had normal serum tyrosine levels. In monthly follow-ups, one of the patients had serum phenylalanine level below 2mg/dl, so his diet was discontinued and later studies showed that his phenylalanine level had remained between 2 and 6 mg/dl.

The incidence of hyperphenylalaninemia was 1/10000. Mild hyperphenylalaninemia has seen in 62.5% of the affected infants and 37.5% cases had classic PKU.

All patients except one had normal growth and development. One patient had recurrent attacks of convulsion accompanied with developmental delay despite serum phenylalanine levels between 2 and 6 mg/dl. After BH4 loading test, one patient had serum phenylalanine level below 2mg/dl which falls into the category of BH4 deficiency (Table 1). Patient no five had a 32% decrease in serum phenylalanine level after 8 hours of BH4 administration who was considered BH4 responsive. Other patients who initially had serum phenylalanine levels above 6 mg/dl did not show any significant change in serum phenylalanine levels after BH4 loading test.

## Discussion

Screening programs for neonates is implemented in Fars province since 2004. This program also includes screening for hyperphenylalaninemia with Guthrie method. Due to lack of other methods, Guthrie method was the first method to propose<sup>[3]</sup>. In this study the serum phenylalanine level of the suspected infants was measured with HPLC method.

In this study the annual incidence of hyperphenylalaninemia was estimated to be 1/10000 in Fars province and 62.5% of the affected infants had mild hyperphenylalaninemia.

In a study by Al Hosani et al in UAE between 1998 and 2000 the incidence of hyperphenylalaninemia was estimated to be 1/20000<sup>[8]</sup>. Another study by De Baulney in France in 2007 showed that the incidence of hyperphenylalaninemia was about 1/10000<sup>[9]</sup>. In 2004 Loeber carried out a study in different European countries and reported a wide range between 1/3000 and 1/30000 for hyperphenylalaninemia<sup>[10]</sup>. In 2004 Zhonghua conducted a retrospective study in China which included infants born between 1985 and 2001, and reported an incidence rate of 1/11144<sup>[11]</sup>.

Present study revealed a percentage of 62.5% for mild hyperphenylalaninemia compared to 37.5% for classic PKU. It seems that most of the

**Table 1:** Results of BH4 loading test in 8 patients with hyperphenylalaninemia

Patients' No.	Ph. A. level at diagnosis (mg/dl)	Ph. A. level before BH4 test (mg/dl)	Ph. A. level 4 hours after BH4 test (mg/dl)	Ph. A. level 8 hours after BH4 test (mg/dl)	Ph. A. 24 hours after BH4 test (mg/dl)
1	21	17.4	19	20	19.8
2	40	17.6	20.5	17.6	19
3	30	20	20	23	24
4	15	7	7	6	10
5	11.4	17	21	11.4	13
6	15	13	12	11	12
7	15	3.9	1.4	0.9	1.4
8	16	16.3	1.5	0.8	0.5

Ph. A: Phenylalanine

BH4: tetrahydrobiopterin



cases of hyperphenylalaninemia in Fars province have mild hyperphenylalaninemia which is in contrast to the percentage reported from other countries. For example, De Baulney reported that 81.6% of his cases of hyperphenylalaninemia had classic PKU and only 17.2% had non PKU hyperphenylalaninemia<sup>[9]</sup>.

Regarding the BH4 loading test, only one out of eight patients responded well to BH4; interestingly this was the same one who had recurrent attacks of convulsion and developmental delay.

Another patient responded partially to BH4, he had mild hyperphenylalaninemia and his serum phenylalanine fell to about 32% after BH4 loading test. These two patients demonstrated the first response to therapy 8 hours after the administration of BH4. Other patients did not show any response at any time of the BH4 loading test. So it seems that the response to BH4 obtained after 8 hours is not so much different from the one obtained after 24 hours.

This result agrees with those acquired by Fiego et al. who followed the serum phenylalanine level of affected infants 48 hours after BH4 loading test and concluded that no more study is needed after 8 hours<sup>[4, 12, 13]</sup>.

This study showed that south of Iran has a high incidence of hyperphenylalaninemia and Guthrie test is a valuable and simple screening test for neonates and justifies its expansion to all parts of Iran. It was also demonstrated that in contrast to previous studies, the incidence of mild hyperphenylalaninemia is greater than classic PKU. This study also shows that it may be cost beneficial to use BH4 loading test routinely for all cases of hyperphenylalaninemia. Regarding the high cost of this drug, if routine prescription is not possible, it is suggested that patients with nervous system problems and developmental delay despite the use of phenylalanine-restricted diet, and serum phenylalanine level between 2 and 6 mg/dl, should be studied for BH4 deficiency and receive the treatment accordingly.

Due to lack of access to petersins and mass tandem spectrometry methods, petersins measurement was not possible and the second sensitive test (BH4 loading test) was performed<sup>[3,4]</sup>.

## Conclusion

This study showed that Fars province (south of Iran) has a high incidence of hyperphenylalaninemia. The high incidence makes a comprehensive screening program for management of the disease necessary.

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**Conflict of Interest:** None

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