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Original Article

Epidemiological and Clinical Study of Phenylketonuria (PKU) Disease in the National Screening Program of Neonates, Fars Province, Southern Iran

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Abstract

Background: Classic phenylketonuria (PKU) is a rare metabolic disorder that results from a deficiency of a liver enzyme known as phenylalanine hydroxylase (PAH). In this study, we researched about PKU epidemiological factors and health quality of patients after the neonatal screening program.

Methods: Neonatal screening for PKU was conducted by one neonatal screening center in Fars Province, in Shiraz Paramedical University. All Fars infants must refer only to this center, in which a heel prick blood sample of each infant was collected at 72 hours postnatal on to standard filter paper and asked questions from the children's parent's and the doctor examined the patients receiving phenylalanine- free milk through examining the children's development. PKU was screened by Fluorometric method.

Results: Totally of 70477 newborns screened for PKU, 15- cases of PKU detected with an incidence of 1:4698. In "Eghlid", that is a city in Fars Province. The prevalence of the disease is 1:382 of newly born babies. The frequency of familial marriage in these children's parents is 86.6%. Twenty nine percent of them were observed among those who had married their close relatives. Mean rate of normal development in PKU patients was 95%.

Conclusion: Consanguineous marriage is a major cause in that pattern particular in Iranian. The treatment of PKU after newborn screening is used. With special diet in above of 90% newborn is satisfactory. Now screening should be executed for all of family that they have familial history of PKU in Iran.

Keywords: *Phenylketonuria (PKU), Screening, Consanguineous Marriage, Mental retardation, Iran*

Introduction

Phenylketonuria (PKU: OMIM#2616001) (1) is an autosomal recessive disorder caused by a deficiency of phenylalanine hydroxylase (PAH, EC 1.14.16.1). PAH catalyses the hydroxylation of phenylalanine to tyrosine. The PAH gene spans about 90 kb on chromosome 12 q 22-12 q 24. Currently, more than 490 PAH mutations have been described (2). PAH mutation frequencies show strong variation among populations. These differences are attributable to the history of the populations (3-8).

The mutation profile of the PAH gene is not restricted to any region, but is spread throughout the structural domains and they present clinical and biochemical phenotype of patients. The active PAH enzyme is composed of 4 monomeric protein (9). PAH is a hepatic enzyme that catalyses the hydroxylation of 1-phenylalanine (1-phe) to 1-tyrosine using tetrahydrobiopeterin (BH4) as a cofactor (10).

The normal rang of phenylalanine is 3.9 mg/dl in newborn. The normal and abnormal ranges of phenylalanine metabolism in plasma and urine are presented (Table 1) (11). If it is precociously introduced, a low-phenylalanine diet prevents the mental retardation associated with PKU (10, 12, 13). Consequently, neonatal screen-

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ing programs have constantly targeted PKU (14, 15).

Determination of the PAH mutations in each patient can help predict the severity of the disease (16-18). The result of PAH mutations is phenylalanine increase in blood and its precipitated it on nerves system and brain (19). The untreated state is characterized by mental retardation, microcephaly, delayed speech, seizures, eczema, behavior abnormalities, and other symptoms.

For PKU, the reported incidence ranges from 1 per 13500 to 1 per 19000 newborns. Approximately 1 of every 15 000 infants in the United States is born with PKU (20).

The incidence of PKU in the U.S, Britain and most of Western Europeans vary between 1 in 11000 and 1 in 15000 births. Etiologically speaking, the specialists studying the PKU carrier gene among Indo-European tribes found a familial relationship, dating back to many years ago, among the carrier. Moreover, they found that this disease was prevalent in Iran and Mediterranean countries (21). The incidence of PKU in Latvia, China, Brazil, London and Portugal are 1:8,700. (22), 1:11144 (23), 1:20000 (8), 1:12000(24) and 1:12037 (25) births.

Because effective treatments exist to prevent symptoms, all countries should be screening infants for PKU. The current treatment for this disorder involves strict metabolic control using a low-Phe diet that includes specialized medical foods. The small amounts of Phe coming from breast milk or commercial infant formula considered sufficient intake in babies. In older children, protein intake is calculated each day, whereby a child is allocated a certain number of grams or units of daily protein, depending upon longitudinal plasma Phe concentrations. Foods such as eggs, milk, cheese, meat, poultry, fish, dried beans and legumes which are high in protein are excluded from the diet (26).

The newborn screening programs for PKU have been remarkably successful: When diagnosed early in the newborn period and treated to achieve good metabolic control, infants have normal health and development and can expect a normal life span.

Then every effort such as screening should be use to start the diet in early infancy if possible with the first 10 days of life (21, 27). Treatment of neonates born with PKU should be initiated as soon as possible but no later than 7 days after birth. Phe levels should be reduced as rapidly as possible.Breastfeeding is encouraged along with Phe-free formula. Because of the need for early initiation of treatment, hospitals should ensure that screening samples are sent for analysis within 24 h of collection and results are returned to responsible parties within 7 d of an infant's birth.

Children treated early for PKU demonstrate subtle problems in cognitive function, school achievement, behavioral adjustment, and quality of life. Related issues concern how early to begin treatment, affects of fluctuations in metabolic control, level of optimal metabolic control, and relaxation of metabolic control. Controversy surrounds these issues. Children with PKU score somewhat lower than expected on IQ tests based on parent and sibling IQs, but their performance is still in the average range.

In this study, we researched about PKU epidemiological factors and health quality of patients after the neonatal screening program.

Material and Methods

Neonatal screening for PKU was conducted by one neonatal screening center in Fars Province. To carry out the present study, the researcher, by referring to the Iranian Society of Child Health Care in Shiraz paramedical university and all Fars infants must refer only to this center, collected data from all the newborn babies from 2000 to 2005, in which a heel prick blood sample of each infant was collected at 72 h postnatal on to standard filter paper and asked questions from the children's parent's and the doctor examined the patients receiving phenylalanine-free milk through examining the children's development. PKU was screened by Fluorometric method.

It was noticed that the normal range of phenylalanine was below 3.9 mg/dl and the abnormal range was above 3.9 mg/dl. The Guthrie test was repeated whenever the phenylalanine range was above 3.9 mg/dl.

Results

Base on the study carried out on 70477 babies born in the Fars Province, 15 babies appeared positive for the PKU out of which 12 out of 53311 cases were those who were born in Shiraz and 3 out of 17166 in other cities of the province. Four out of 15 patients died and 11 survived.

The frequency of various variables is as follows: 57.19% of these children's fathers were under 35 yr of age while 42.86% of their mothers were under 30.

Familial marriage frequency among couples with 3 to 4 degrees of consanguinity each was 33.3%. Among the Fars tribe, the highest rate of familial marriage was 71% while among the Lore, it was about 21%.

There was no significant relation between the presence of PKU and smoking mothers or those exposed to x-ray, chemical dugs, etc.

Twenty nine percent of the PKU was observed among those who married their close relatives.

Mean and frequency of the amount of plasma phenylalanine among the PKU patients was as follows: 3.9-10 mg/dl (33%), 10-20 mg/dl (53.3%) and over 20 mg/dl (13.3%).

Table 1: Normal and abnormal phenyl

Metabolite	Plasma (mg/dl)		Urine (mg/dl)	
	Normal	PKU	Normal	PKU
Phenylalanine	1-2	15-63	30	300-1000
Phenyl pyrovate		0.3-1.8		300-2000
Phenyl lactate				240-550
Phenyl acetate				Raising
Phenyl acetyl glutamine			200-300	2400

Table 2: The effect of phenylalanine on the children's physical and mental growth rate

Effect of phenylalanine	Number of PKU (%)
> 90%	2(50)
80%-90%	1 (25)
50%-80%	0 (0)
<50%	1 (25)

Discussion

The primary factor to be considered in the present study is to find the proportion of the number of babies born afflicted with PKU to all those who have been born alive in Iran (Fars Province). The prevalence of the disease is 1: 4698. This may be due to the fact that the number of consanguineous marriages in the Mediterranean areas in general (and Iran in Particular) outnumbers those of European. This is supported by the studies have been carried out on the prevalence of consanguineous marriage in Iran. Consanguineous marriage is a major feature of family systems in south west Asia. The current prevalence and patterns of consanguinity in Iran as assessing the associated requirement for genetic counseling services for example in one study in Iran a multi stage sampling design was used with a representative total sample of 306343 couples , the overall rate of consanguineous marriage was 38.6% with a mean inbreeding coefficient (alpha)

of 0.0185. First cousin marriages (27.9%) were the most common from of consanguineous union (28).

This disease, in fact, is inherited as the dominant autosomal. Hence, in consanguineous marriage, mainly because of the presence of the higher inbreeding coefficient, the probability of the emergence of PKU may increase.

The other point to be investigated is to find the prevalence of this disease in the tribe or the subpopulation in certain geographical areas. It has been noticed that in Eghlid (a small city in Fars Province) the prevalence of the disease is 1: 382, which is the highest compared to the other subpopulation residing in Fars province. This can be attributed to the prevalence of PAH pathogen gene in this area as a "founder effect ". Furthermore, due to the familial marriage, which is very common there, the disease is more prevalent in this area. Concerning the age of the parents, mothers are mainly under 30 and fathers under 35 yr old, indicating no correlation between parents' age and the presence of the disease.

Moreover, the number of pregnant women being exposed to the pathogenic agents (such as X-ray, drug, chemical agents, etc.) is statistically insignificant. This reinforces the point that there is no relation between the presences of the disease with the presence of pathogenic agents in the neighboring environment.

It is worth mentioning that 4 out of 15 patients with the average 13mg/dl phenylalanine died during their infancy. However, due to the inaccessibility of the medical records, we could not find factor underlying their death; that is whether this was due to the fatal PKU or other unknown.

Concerning the amount of phenylalanine of plasma, it was notice that 86.7% of the afflicted children had phenylalanine less than 20 mg/dl and 13.3% more than 20mg/dl. It is worth mentioning that this is not in line with findings in the USA, in which, according the studies carried out, 85.1% of the afflicted children had phenylalanine more than 20 mg/dl (29) .

The difference may be attributed to the number of the patients in Iran, which was very low or due to the difference in their mutation type and the inherence of impairment quantitatively, such as delay in their development, which is one of characteristic biological differences in the population.

Evaluating the disease progression and the effect of screening after birth of the children using phenylalanine-free milk strongly supports the importance and effectiveness of screening. Studies performed in other countries (1953) reported that the IQ of 85% of patients not receiving phenylalanine in their diet was under 40 and 37% under 10 and 1% under 70 (30) . In the following years, however, the findings of the studies showed a significant increase (95%) in the IQ of the patients, receiving phenylalanine from childhood, which was normal or almost normal (31).The present study revealed that 50% of the children enjoyed a more than 90% of normal growth, 25% diverted about 10-20 %from the normal growth while in the rest of the population (25%) only 2-5% of normal growth was noticed (Table 2) .

It is pertinent to mention that children of the first group (with more than 90% of the normal growth or more) all were under 6 months who received the dietary milk, while those under 50% of normal growth were those above 6 months who received dietary milk in addition to some supplementary food. This may not be due to the fact that the type of gene mutation and the emergence symptoms among different age group of PKU children were different accompanied their diet (32).

When children were examined to evaluate their mental growth , physical growth and loco motor development the researcher noticed that the more the parents follow the doctor's prescription concerning the children's diet, the healthier and the closer the children were to the normal growth. Of course, various studies have shown that this is not enough. That is to say, learning problem among PKU patients even those who underwent appropriate treatment persists. Hence,

it is assumed that other factors such as the reduction of neurotransmitters, leading to insufficient transmission Tyrosine from neurons' cell membranes (33). In other words, hypotyrosinemia in these patients accompany learning difficulty.

In conclusion, genetic testing for PKU has been in place for almost 40 yr and has been very successful in preventing severe mental retardation in thousands of children and adults. Metabolic control is necessary across the lifespan of individuals with PKU. A comprehensive, multidisciplinary, integrated system is needed to delivery of care to individuals with PKU. Greatly needed are consistency and coordination between screening, treatment, data collection, and patient support programs. There should be equal access to culturally sensitive, age-appropriate treatment programs. Ethically sound, specific policies for storage, ownership, and use in future studies of archived samples remaining from PKU testing should be established. Research into the pathophysiology of PKU and relationship to genetic, neural, and behavioral variation is strongly encouraged. Uniform policies must be established to remove financial barriers to the acquisition of medical foods and modified low-protein foods and to provide access to support services needed to maintain metabolic control in individuals with PKU. Research on nondietary alternative treatments for PKU is strongly encouraged. To achieve optimal statistical power and cross-cultural applicability, it will be beneficial to use data acquired via national and international collaboration. phenylketonuria, hyperphenylalanimia, phenylketonuria screening, phenylalanine-restricted diet, maternal phenylketonuria, newborn screening, phenylalanine monitoring, and phenylketonuria outcomes.

To avoid the occurrence of this disease, it is pertinent for the familial marriage in the family in which there has been at least one case of the disease to have genetic counseling prior to their marriage, before and during the pregnancy. Such consultation is required not only for the prospected couples whose families have such a patient but also for their relatives. In other words, continuous genetic counseling complements the screening. Moreover, the patient after being screened and identified should be closely observed to receive appropriate treatment as well as phenylalanine-free milk and special diets. Hence, it seems necessary to found some institutions supporting PKU patients.

Finally, it is recommended that doctors examining patients receiving phenylalanine-free milk closely observe the children's physical, emotional and social growth and report the findings statistically.

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