Original Research Article

Annual incidence of phenylketonuria in Sulaimani City

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Abstract

Phenylketonuria (PKU) is a hereditary, autosomal recessive disorder characterized by severe hyperphenylalaninemia which if untreated, would invariably result in the development of signs and symptoms of classic PKU with profound intellectual disability. Newborn screening in Sulaimani city had started in 2013 for screening of (PKU), congenital hypothyroidism and G6PD deficiency. The purpose of the present study was to find out the annual incidence of PKU in Sulaimani city. All neonates born in Sulaimani city including all hospital deliveries (both private and public governmental hospitals) in addition to the home deliveries that were referred to (Registration Bureau of Births and Deaths) during the period January 1st to December 31st 2014 were included. A heel prick blood sample was taken from neonates and sent to Sulaimani Pediatric Teaching Hospital laboratory where phenylalanine level were estimated by ELISA method on the dried blood spot. From all the screened neonates (8,255 cases) who were born in Sulaimani city, 11 cases were found to have a high level of serum phenylalanine from the first blood sample and after subsequent serum level assessment only one case was proven to have phenylketonuria and referred to nutritional rehabilitation center for therapy and follow up. The annual incidence of phenylketonuria in Sulaimani city was found to be 1.2 in 10,000 neonates.

Keywords: Phenylalanine, phenylketonuria, incidence, Sulaimani

INTRODUCTION

PKU was first described by Asbjorn Folling, one of the first Norwegian physicians to apply chemical methods to the Study of medicine in 1934 (Gonzalez et al., 2010), from which eventually came the rationale for early diagnosis by newborn screening.

Phenylalanine (Phe) is an essential amino acid and dietary phenylalanine not utilized for protein synthesis is normally degraded by way of the tyrosine pathway. Deficiency of the enzyme phenylalanine hydroxylase (PAH) or of its cofactor tetrahydrobiopterin (BH4) causes accumulation of phenylalanine in body fluids and in the brain (Iraj and Melvin, 2011).

Complete enzyme deficiency, in which serum Phe concentration exceeds 20 mg/dl will cause classical or severe PKU (Guttler, 1980), while persistent hyperphenylalaninemia (HPA) is a milder form of the disorder in which there is only a partial reduction in the activity of the PAH enzyme from 2 to 5% of normal. Residual enzyme activity causes mild PKU (Phe concentration 10 to 20 mg/dl) and HPA (Phe concentration 2.5 to 10 mg/dl) (Guttler, 1980; Ushakova et al., 1997).

The gene coding for PAH enzyme, an enzyme with a key role in phenylalanine metabolism pathway, is 90 kbp long and is located on q22–q24.1 regions of chromosome 12. This gene consists of 13 exons and 12 introns (Williams et al., 2008). More than 400 mutations, including deletions, insertions, splicing defects, missense and nonsense mutations, have been identified (National Institutes of Health Consensus Development Panel,
2000). No single mutation predominates in white populations, although certain mutations are more common in specific ethnic groups (Wappner, 2006).

In untreated patients, the hallmark of the disease is intellectual disability (ID) (Fölling, 1934). Mental impairment worsens during myelination in early childhood with increasing dietary exposure, but stabilizes when brain maturation is complete (Erlandsen and Stevens, 1999). The mechanism by which the elevated concentration of Phe causes ID is unknown. Excessive Phe is thought to interfere with brain growth, myelination, and neurotransmitter synthesis (Paine, 1957).

The early diagnosis of the disease, at least within the first days of birth, is a critical measure for prevention. In this regard, neonatal screening has been widely recognized as a fundamental public health intervention in the past fifty years (Guthrie and Susi, 1963).

United States Preventive Services Task Force (USPSTF) recommended newborn screening as an “A” category being able to demonstrate to show a high certainty of benefits (US Preventive Services Task Force, 2008).

PKU is presumed to be more prevalent among the neonates of consanguineous marriages. High incidence of relative marriages is directly associated with a high prevalence of the disease in the population (Nyhan et al., 2005; Jain et al., 1993).

Because of the often gradual development of clinical manifestations, HPA is usually diagnosed through mass screening of newborn infants. In infants with positive results from the screen for HPA, diagnosis are further confirmed by quantitative measurement of plasma phenylalanine concentration. The bacterial inhibition assay of Guthrie (Andermann et al., 2008), which was the first method used for this purpose, has been replaced by more precise and quantitative methods (fluorometric and tandem mass spectrometry). The method of choice is tandem mass spectrometry (MS/MS), which identifies all forms of HPA with a low false-positive rate, and excellent accuracy and precision. In addition, tandem mass spectrometry can identify many other inborn errors of metabolism in a single sample. The national institute of health (NIH) Consensus Development Conference on PKU had recommended mutation analysis and genotype determination on all affected patients for initial diagnosis, genetic counseling, follow-up, and long-term prognosis (National Institutes of Health Consensus Development Panel, 2001).

To the best of our knowledge, the most efficient prognosis of the disease is to control phenylalaninemia which should be done before the end of the first month of birth (Eshraghi et al., 2010). The mainstay of therapy in PKU is dietary restriction of phenylalanine. This requires the use of medical foods including phenylalanine-free protein substitutes (amino acid mixtures or protein hydrolysates) that supply approximately 75 percent of protein requirements (except Phe) (MacDonald, 2000).

Treatment should be initiated as soon as possible, usually before one week of age and Continuation of dietary restriction throughout life appears to be necessary for optimal outcomes (Koch et al., 2002; Bosch et al., 2007). The goals of lifelong nutrition therapy include normal physical growth and neurocognitive development, maintenance of adult health, and normal gestational outcomes in pregnant women with PAH deficiency (Abadie et al., 2005).

Patient and methods

This retrospective study was done in collaboration with neonatal screening department in Sulaimani Pediatric Teaching Hospital that adopted neonatal screening program for all births inside the city in June, 2013 and as the first stage for only births inside Sulaimani city and hoping to expand the program to districts within Sulaimani governorate in the near future.

All newborns from the city visiting (Registration Bureau of Births and Deaths), for the purpose of issuing birth certificate and receiving BCG vaccination were included. A blood sample was taken through heel prick and all samples were transferred to Sulaimani Pediatric Teaching Hospital for neonatal screening department and screened for phenylketonuria, G6PD deficiency and congenital hypothyroidism.

In this study the newborns that were referred to (Registration Bureau of Births and Deaths) from Jan.1st to Dec.31st 2014 were included. There were 8,255 newborns. Neonates underwent screening study for HPA using (quantitative enzymatic assay of Phe by ELISA method on dried blood spot) within 3 to 10 days after birth by collecting blood sample from the corner of infant's heel by experienced technician.

Babies that were excluded in the screening program included:
1. Sick babies who were asked to postpone their visit to Registration Bureau.
2. Babies of the refugee families.
3. Babies outside Sulaimani city (i.e districts and towns outside of Sulaimani governorate) not included.

Blood samples were collected at registration bureau center on a collection card (Whatman903, where in Iran, proper citation of manufacture and location) according to the instruction of manufacturing company, later samples were transferred to Sulaimani Pediatric Teaching Hospital/newborn screening department in a special container. Quantitative enzymatic assay of Phe in dried blood spots were performed using Neo-PKU Kit, Iran made by PISHTAZ TEB Company and ELISA plate shaker.

Determination of the Phenylalanine concentration was done for each sample on standard curve. Results of a typical standard run of a neonatal Phenylalanine are shown in table 1 and figure 1.

The cut off value in neonates was 4 mg/dl. For those
samples in which the Phe value was more than 4 mg/dl concentration the test was repeated in duplicate sample. If still the readings were still more than 4mg/dl the family was recalled to Sulaimani Pediatric Teaching Hospital / newborn screening department and another heel blood sample collected for reassessment. If the Phe level was still more than 4mg/dl, then baby/patients was regarded as having HPA. These cases were referred to the pediatric dietitian and general pediatric specialist for further assessment and management as soon as possible. Because of unavailability of other confirmatory investigations, 3ml of venous blood sample was taken and sent to (NeoLab, Athens, Greece), where serum Phe and Phe/Tyrosine ratio were measured using high performance liquid chromatography (HPLC) method to see whether still the levels were above normal to regard the patient as confirmed PKU. However, patients that were called back for subsequent level assessment in hospital and their serum phe level returned to normal were considered as transient hyperphenylalaninemia.

**RESULTS**

Out of 8.255 included in this study, the first prick blood sample analysis showed that 11(%0.133%) of those neonates had a significantly high level of phenylalanine (more than 4mg/dl) which required further investigations in the hospital, 6 of them were females and 5 were males. Serum phenylalanines of those 11 cases were as follow: 6 cases were between 4.0 and 5.0 mg/dL, 2 cases between 5.1 and 6.0 mg/dL, 1 case was 7mg/dL and 2 cases were more than 7.1mg/dL. Parents of all 11 cases were called to come back to the hospital for further evaluation. On the second estimation of serum phenylalanine 9 cases were proved to have normal serum phenylalanine.

Serum phenylalanine of two cases were still high on the second assessment, one of them was a result of consanguineous parent while other one have non consanguineous parent. The one from consanguineous parent was female with first serum phenylalanine level of 19.1mg/dL and subsequent serum assessment was also high and confirmed to have phenylketonuria and referred to nutritional rehabilitation center to start dietary treatment (Phe free formula which is available in hospital (PKU1 mix formula, milupa company, turkey made).

While the other one was male from non-consanguineous parent with a serum phenylalanine level of 9 gm/dl which had returned to normal before starting
dietary treatment that is why diagnosed as transient hyperphenylalaninemia.

The annual incidence of PKU in Sulaimani city was considered to be 1.2 in 10,000 neonates.

DISCUSSION

Screening for congenital metabolic diseases is an important form of prevention in Pediatrics. This activity is very useful in the detection of many inborn diseases. It should be noted that many kinds of congenital defects can be successfully treated if early detection is made.

Neonatal screening program was implemented in Sulaimani starting in June 2013. If we compare our country with many countries at the regional level, we are behind this practice. Neonatal screening program was started in California in 1966, the first year for compulsory newborn screening in California (Peterson et al., 1968), and in United Kingdom in 1964. To our knowledge, Sulaimani Pediatric Teaching Hospital was the first in Iraq to launch this program.

Nutritional rehabilitation center is the only center in Sulaimani city that receives cases diagnosed as phenylketonuria. During the period of our study throughout 2014 only one case was registered. The case was diagnosed by neonatal screening program in Sulaimani city, this supports that no cases were missed by our screening program.

Many studies have been done throughout the world to measure incidence of phenylketonuria in ethnically and genetically very heterogeneous populations, reflected the variations of reported PKU incidences (Zschocke et al., 2003; Jeran et al., 2007; Kremensky et al., 2000).

Unfortunately inside Iraq until 2013 no neonatal screening program had been launched, in other governorates in Iraq other than Sulaimani city, phenylalanine level assessment is done only for diseased children with suspicion of PKU (Rabab, 2013).

In the regional countries like in Iran, there have been many studies on the incidence of phenylketonuria; a study performed by Kabiri and Farhud on 8633 newborns born in different hospitals in Tehran, the Incidence rate was calculated as 1.1 in 10,000 (Farhud and Kabiri, 1982), in another study also in Iran the incidence of phenylketonuria in Fars province was 1.6 in 10,000 (29), which is also similar to Farhud study. If we compare both studies with our results, we see notable similarities, i.e. an incidence rate of 1.2 in 10,000. Possible explanation of this similarity is probably because of two reasons, first we have used machine and kits materials of Iranian origin and the second reason is probably because of similar ethnicity of both populations as Fars and Kurdish are from Iranian descent (Indo-European). If we look at the data of Asadollah study (Asadollah et al., 2010), we will see that 0.04% of cases have transient hyperphenylalaninemia which is nearly similar to the result of this study 0.013% and this support above reasons. Another reason is that the above mentioned studies, like this study, considered serum Phe level of 4mg/dl as the cutoff point for referral and further investigation.

Another important factor that affects incidence rates of phenylketonuria in a population is the rate of consanguineous marriage. For example, in the Iran province of Yazd which is known to have a high rate of consanguineous marriage, one study shows higher incidence of phenylketonuria which was estimated to be 1: 5532 (Ordooei et al., 2015). Another example of a community with a high rate of consanguineous marriage and high incidence of phenylketonuria is Turkey with incidence of 1: 2600 (Scriver and Kaufman, 2001). The incidence of PKU in Europe in 2004, was variable between countries and the range was between 1:3000 and 1:30000 (Loeber, 2007).

Examples of incidence in few European countries are like follows; France 1: 13500, United Kingdom 1: 14300, Italy 1: 17000(32), low incidence of phenylketonuria is probably because of low consanguineous marriage rate.

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CONCLUSION

In conclusion the incidence of PKU in Sulaimani city is relatively high, therefore neonatal screening should be maintained and extended throughout the region.

REFERENCES


Gutierrez F (1980). Hyperphenylalaninemia Diagnosis and classification of the various types of phenylalanine hydroxylase deficiency in childhood. Acta Paediatr Scand ; 280, 1-80


