Incidence of Neonatal Phenylketonuria in Hormozgan Province, Southern Iran, 2014-2016

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Abstract

Background and Aim: Phenylketonuria is a hereditary, autosomal recessive disease characterized by deficiency of hepatic phenylalanine hydroxylase. Dietary PKU treatment and regular monitoring of blood Phe are responsible to prevent symptoms. Therefore, present research was performed with the objective of determining incidence of PKU in the Hormozgan Province, South of Iran. Material and Method: This cross sectional study was carried out in Bandar Abbas, Hormozgan among 71677 neonates, who were born from March 21, 2014 to March 21, 2016. The newborns were screened during the days 3 to 5 after birth. Finally, the incidence rate was calculated and statistical analysis conducted by Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc, Chicago, IL, USA). Results: Among study population, 36705 (51.2%) of cases were male and 34972 (49.8%) were female and 15 cases (12 in first year and 3 in second year) were ELISA positive (considered as suspected PKA) and referred for confirmatory HPLC test. Finally, three neonates were confirmed as PKA positive cases by HPLC test. Overall incidence rate of neonatal PKA in two studied years was 1:23866. Conclusion: We can conclude that the incidence rate of phenylketonuria in southern Iran is reduced to 1:16987 in 2015 and 1:37736 in 2016, which is probably due to improved knowledge of general population of Iran about controllable determinants of PKU.

Keywords: Phenylketonuria, Newborn, Incidence, Iran.

Introduction

Phenylketonuria is a hereditary, autosomal recessive disorder caused by hepatic phenylalanine hydroxylase or its cofactor tetrahydrobiopterin deficiency, characterized by elevated serum levels of phenylalanine and its metabolites including phenylketones [1]. Deficiency of hepatic phenylalanine hydroxylase leads to classic phenylketonuria (PKU), while deficiency of tetrahydrobiopterin causes malignant PKU [2]. Neonates with PKU appear normal at birth. However, elevated phenylalanine in untreated cases causes gradual development of irreversible brain damages such as mental retardation, microcephaly, delayed speech, and seizures and hypopigmented skin and hair [3]. Currently, the manifestations of phenylketonuria are rarely reported in developed communities due to early detection by newborn screening and successful treatment with a low phenylalanine diet. In addition to dietary PKU treatment, regular monitoring of blood Phe levels is mandatory at least throughout childhood, adolescence, and in pregnant women [4]. Therefore, due to existence of effective therapies to prevent symptoms, all populations are responsible to screen neonates for PKU [5]. Based on the previous studies, more than 490 phenylalanine hydroxylase mutations have been involved in PKU. Results of PAH mutations are phenylalanine elevation in serum and its sedimentation on nerves system [6]. The mutation frequencies indicate strong variation among populations. For PKU, the published incidence ranges from 1
per 8700 to 1 per 120000 newborns [7]. According to the literature, the incidence of PKU is approximately higher in developing countries than in developed countries. For example, the value in Iran has been reported to be 1 per 10000 by two studies and 1:4000 by another report, while in Latvia, USA, China, Sweden, Brazil, Scotland, London, Japan, and Portugal, it was 1:8700, 1:13000, 1:11144, 1:38000, 1:20000, 1:60000, 1:12000, 1:120000, and 1:12037 respectively [4, 8-15]. Awareness of incidence of PKU in various geographical regions or different periods of time is important mainly due to the planning and foresight of healthcare authorities and relevant organizations [15]. In this regards, very scarce and old data exist on PKU management in Iran, especially Hormozgan Province in the South. Thus, present investigation was carried out with the objective of determining incidence of PKU in the Hormozgan Province, South of Iran to identify the involved newborns for further complications and medical expenses.

Materials and Methods

Study Design and Participants

This cross sectional study was conducted in Bandar Abbas, Hormozgan (located in the South of Iran) with the cooperation of Hormozgan University of Medical Sciences. A total of 71677 neonates, who were born from March 21, 2014 to March 21, 2016 (two complete Iranian calendar year), were recruited in the study by consecutive sampling.

Parents of newborns were informed about the aim of this survey and confidentiality of personal information and after obtaining informed consent, they were included in the present investigation. Exclusion criteria were lost of follow up, lethal anomaly, and dissatisfaction of parents to participate in this survey.

Study Procedure

During the days 3 to 5 after birth, the newborns were screened through a mandatory neonatal screening program. In fact, heel blood samples were taken on S and S 903 Guthrie papers (Schleicher and Schuell 903, Bioscience, Germany) by experienced technicians. The samples from 13 cities of Hormozgan Province (including Qeshm, Jask, Hajjiabad, Bandar Abbas, Bastak, Bandar Lengeh, Bandar Khamir, Abu Musa, Rudan, Minab, Parsian, Sirik, and Bashagard) were sent to screening laboratory of health authorities of Bandar Abbas city, where the serum phenylalanine were assessed by the ELISA method. Additional data including demographic characteristics, history of related risk factors, and results of physical examinations were recorded for each participant in a pre-designed data collection sheet. Those who had phenylalanine levels above 2 mg/dl in screening test were referred for re-evaluation by high performance liquid chromatography (HPLC) method. For this technique, 3 mL of venous blood were obtained from the suspected newborns and if the serum phenylalanine levels were equal or more than 10 mg/dL, the newborns were considered as positive cases. The cases with serum phenylalanine levels between 4 to 9.9 mg/dL were refered for another blood sample test for one week later. If the serum phenylalanine levels were reduced to <4 mg/dL, they considered negative/healthy cases, whereas if the serum phenylalanine levels were again equal or more than 4mg/dl, they were considered definitely as positive samples. Defaced samples were considered as samples with small size due to inappropriate sampling method, while unacceptable samples were considered as very large samples that were in contact with the side samples or fungus and mosquito ridden and eaten them, respectively. Re-sampling was performed for both defaced and unacceptable samples.

Study Outcome

The incidence rate is the number of new cases per population at risk in a given time period. This parameter was considered as study outcome and calculated by following formula for each year of present investigation period.

\[
\text{Incidence Rate} = \frac{\text{Number of new case of disease in a given time period}}{\text{Total person time at risk during the study period}}.
\]

Statistical Analyses
Statistical analysis was conducted by Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc, Chicago, IL, USA). The data are expressed as number (N) and percentage (%). Chi-square test was used to compare the categorical variables and a Student’s t-test was used to compare continuous variables between the two groups. A two sided α = 0.05 was considered statistically significant.

Results

Total 81219 neonates were enrolled while 71677 were screened, 33973 in first year (from March 21, 2014 to March 21, 2015) and 37704 in second year (from March 21, 2015 to March 21, 2016) of present survey, and no participants were excluded from the study during the research period. Defaced and unacceptable samples were 5922 and 4328 samples, respectively.

There was no statistically significant difference between the positive and negative groups in respect of demographic and baseline characteristics, including gestational and admission ages, birth weight, and gender (p>0.05).

Among this population, 36705 (51.2%) of cases were male and 34972 (49.8%) were female and 15 cases (12 in first year and 3 in second year) were ELISA positive (considered as suspected PKA) and referred for confirmatory HPLC test. Therefore, complete demographic characteristics and results of confirmatory HPLC test of suspected neonates are shown in table 1 and 2, respectively.

Table 1: Complete demographic and baseline characteristics of the suspected neonates

<table>
<thead>
<tr>
<th>Variables</th>
<th>No(%)</th>
</tr>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5(33.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>10(66.7%)</td>
</tr>
<tr>
<td>Positive familial history</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Consanguineous marriage</td>
<td>8(53.3%)</td>
</tr>
<tr>
<td>Habitat (city)</td>
<td></td>
</tr>
<tr>
<td>Qeshm</td>
<td>1(6.7%)</td>
</tr>
<tr>
<td>Jask</td>
<td>1(6.7%)</td>
</tr>
<tr>
<td>Hajjiabad</td>
<td>1(6.7%)</td>
</tr>
<tr>
<td>Bandar Abbas</td>
<td>2(13.3%)</td>
</tr>
<tr>
<td>Bastak</td>
<td>1(6.7%)</td>
</tr>
<tr>
<td>Bandar Lengeh</td>
<td>3(20%)</td>
</tr>
<tr>
<td>Bandar Khamir</td>
<td>3(20%)</td>
</tr>
<tr>
<td>Bashagard</td>
<td>1(6.7%)</td>
</tr>
</tbody>
</table>

Table 2 Results of confirmatory HPLC test of suspected newborns

<table>
<thead>
<tr>
<th>Value</th>
<th>No (%)</th>
</tr>
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<tbody>
<tr>
<td>&lt;4 mg/dL</td>
<td>12(80%)</td>
</tr>
<tr>
<td>4-19.9 mg/dL</td>
<td>2(13.3%)</td>
</tr>
<tr>
<td>20≤ mg/dL</td>
<td>1(6.7%)</td>
</tr>
</tbody>
</table>

As shown in table 2, three neonates were confirmed as PKA positive cases by HPLC test. Two of them (one girl from Bandar Abbas and one boy from Qeshm) were diagnosed in first year of research, while another case was a boy from Bashagard in second year of study. Of these confirmed newborns, two had positive history of parental consanguineous marriage without any positive familial history. Incidence of neonatal PKA was calculated for each year by the mentioned formula, and the results were 1:16987 and 1:37736 in the first and second years, respectively. Overall incidence rate of neonatal PKA in two studied years was 1:23866.

Discussion

Newborn screening for PKU in Iran is a nationwide program which covers newborns around the country. In present research, among 39644 babies born in first year, 33973 cases (85.70%) conducted screening tests while in second year, 37704 neonates (90.69%) among 41575 birth, performed mentioned assays. The probable reason of this increase was some facilitating and persuasive factors established by healthcare
authorities in second year of present investigation (16). Based on baseline genetic principles, autosomal recessive diseases such as PKU are more common in boys due to the transition from carrier mothers. The results of our survey were also in agree with the literature and genetic principles (two boys vs one girl) [17].

As described in results section, the suspected cases in first year of screening were 4 times (12 vs 3) more than that value in second year of study. This significant difference was mainly due to the increase of cut off point from 3.5 to 4 in second year [18]. During the research period and confirmatory tests, 3 cases of PKU were detected and the overall incidence of PKU was 1:23866 in these two years. Based on large, multi center studies in the world, the incidence of PKU in other countries ranges from 1:8,700 to 1:120,000. The highest value was observed in Latvia, while the lowest incidence was in Japan [4, 8-15]. PKU is a hereditary disease, mainly caused by a genetic mutation in the human phenylalanine hydroxylase (PAH) gene. There are many various types of mutations that can adversely impact on PAH gene. In order to have PKU, a person must inherit the abnormal gene from both parents [19]. There are two major types of determinants for PKU incidence, containing controllable such as consanguineous marriage and genetic counseling and uncontrollable factors such as race and ethnic. For example, the value is much more common in whites than in other racial groups [20]. Among European countries, average incidence of PKU was significantly lower than developing countries in Middle East such as Iran. This significant difference is attributed to both types of determinants (controllable and uncontrollable) [21]. For instance, in developing countries with low level of knowledge and high percentage of rural life, consanguineous marriage is more prevalent. In this event, mainly due to the presence of the higher inbreeding coefficient, the probability of the incidence of PKU may increase [22].

There have been limited studies on the incidence of phenylketonuria in Iran. Due to ethnic and populace similarities between provinces of Iran, we can compare our results to other regions of the country [23]. In a small study performed by Golbahar et al. on 1544 neonates born in different hospitals in Tehran, the incidence of phenylketonuria was calculated to be 1:3672 [12]. This was unusually high, indeed one of the highest reported incidences in the world. In this regards, Senemar et al. screened 70477 newborns for PKU in 2008 in Iran. The findings illustrated 15 cases of PKU with an incidence of 1:5000 [10]. About 2 years later, Habib et al. conducted a similar study in Iran with a population of 4336878 cases in Fars Province. In accordance to their data analyses, the incidence rate of phenylketonuria in Iran was reduced to 1.6 in 10000 [8]. Thus, a pleasant trend was found in the incidence rate of neonatal PKU in Iran in passing of time. In line with this explanation, findings of our survey (1:16987 and 1:37736 in the first and second years, respectively) confirm this progressive trend. This may be due to improvement in mentioned controllable variables including consanguineous marriage and genetic counseling. In fact, knowledge of the people and rural life were elevated and decreased, respectively in regards to mentioned topics, in recent years [24]. Therefore, decreasing trend of consanguineous marriage and increasing trend of genetic counseling are two main prophylactic factors in Iran [25]. However, wide investigations are required to document and confirm those trends of consanguineous marriage and genetic counseling in Iran by comparing with previous surveys. Finally, we can conclude that the incidence rate of phenylketonuria in southern Iran is reduced to 1:16987 in 2015 and 1:37736 in 2016, which is probably because of improved knowledge of general population of Iran about controllable determinants of PKU [26-34].

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**Author Contributions**

Study concept and design:farzaneh dehghan. Analysis and interpretation of data: Nader zolghadri, and Yasaman Saadatpouri. Drafting of the manuscript:farzaneh dehghan. Critical revision of the manuscript for important intellectual content: Sepehr Rasekhi. Statistical analysis: Nader zolghadri.
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