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ORIGINAL ARTICLE

Descriptive Analysis of Phenylketonuria (PKU) in Newborn Screening test in Ardebil Province

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ABSTRACT
PKU is an inherited metabolic disorder caused by a deficiency of essential amino acids phenylalanine catabolism that lead to the increased concentration of phenylalanine in the toxic range. This condition has irreversible effects on brain development that can include mental retardation and severe developmental delay. The high prevalence of this disorder among Iranian ethnic group had been shown. In recent years, systematic screening program in Iran provinces is the major reason of increased the number of infants identified with PKU and effective treatment strategies caused to reduces the development retardation and complications of PKU in infants. At the first 72 hours after birth, of all children a blood sample taken from the heel and send to the laboratory for testing to Guthrie immediately. Then the test repeated for the positive results infants. Then the positive repeated samples send to Pasteur Institute of Iran for test further and reject the malignancies. Of the 50506 births in 24 months in Ardebil province 16 cases with phenylketonuria were detected by screening. Among them 62.5 % of the patients had the classic phenylketonuria and 37.5 % had mild phenylketonuria. The prevalence of phenylketonuria in Ardebil was 1:3163. The prevalence of consanguineous marriages among patients was 81%. Given the high rate of intermarriage among patients seems to be a necessary requirement for genetic counseling before the marriage. Besides raising awareness and knowledge in relation to genetic disorders can have positive results in reducing the incidence of this disease.
Key words: *Phenylketonuria, screening test, Ardebil, Iran*

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INTRODUCTION

Phenylalanine essential amino acid is converted to tyrosine by liver enzyme of Phenylalanine Hydroxylase (PAH) and in presence of Tetrahydrobiopterin (BH4) cofactor [1]. Fouling for the first time in 1934 found that special types of mental disabilities are in relation with phenylalanine level in body fluids; he named this state as Phenylpyruvic oligophrenia but it was very quickly changed to Phenylketonuria [2]. Then it was determined that defect in PAH enzyme is caused classic phenylketonuria disease and defect in those enzymes that provide cofactor tetrahydrobiopterin (BH4) for PAH also caused non-classic phenylketonuria. These enzymes include *Dihydrobiopterin Synthetase and Dihydrobiopterin Reductase* [3, 4].

Different classifications have been presented for phenylketonuria but the most famous of them is Guldberg *et al*. [5] classification which divides the shortage of PAH to 4 groups according to the phenylalanine tolerance level in aged less than 5 years children:

1- Classic phenylketonuria: it is created in the complete lack of PAH activity. Patients can tolerate dietary phenylalanine about250-350 ml/day to keep phenylalanine plasma concentration less than 5 ml/dl and if these patients do not receive food regimen, they will stricken with mental severe and irreversible disabilities.
2- Average Phenylketonuria: stricken babies can tolerate the proportion of 350-400 ml of phenylalanine in their daily dietary.
3- Mild (low) Phenylketonuria: stricken babies can tolerate the proportion of 400-600 ml of phenylalanine in their daily dietary.

4- Mild hyper-phenylalanine: when stricken babies take normal dietary, they have phenylalanine plasma concentration less than 10 ml/dl.

Several studies have been accomplished throughout the world related to Phenylketonuria prevalence [1] that different prevalence level has been seen in various geographical regions, in population with different culture and in various races. It has been mentioned in some research that phenylketonuria patients are lack of special clinical pretenses in the first few months of their life [11] that this issue increases the value of doing screening in all babies.

Increasing of phenylalanine concentration to higher than its normal values has the most side effects on brain evolution and leads to mental retardations, IQ decrease to lower than 50, microcephaly, epilepsy and etc.; almost all of these side effects and problems are dramatically preventable by identifying of babies and controlling of phenylalanine level in their dietary [12, 13].

The most frequent clinical pretenses in Iran have been mentioned in different studies which are perceivable in summary at Table 2.

It has been observed in several various studies that curing children stricken with phenylketonuria with appropriate and controlled dietary in the first two weeks of life has led to eradicating/obviation of disease intense side effects and also normal growth and development in them [14, 15].

Intermarriages are one of the most common causes of phenylketonuria in different countries that have a strong relationship with cultural roots of each nation. The relationship between intermarriages with phenylketonuria has great important in Iran and there have been reported in different studies the phenylketonuria prevalence and its relation with being relative of patient's parent. This information has been mentioned at Table 3.

Since the frequent of intermarriages is high in Ardabil province, considering the prevalence level of phenylketonuria as a hereditary disease and its mode of inheritance in order to notifying and also presenting precautionary approach are the most important purposes of this research.

MATERIAL AND METHODS

The screening program of born babies is broadly and comprehensively done from October of 2013. In this study, all information related to born babies in 2013-2014 have been collected and compared with other available statistics in Iran and other countries.

It was taken blood sample from the heel of all babies in the first 72 hours after birth (accordance with those conditions that have been mentioned in laboratory standards set of phenylketonuria program at QC program) on filter paper and sent for considering to the laboratory.There were also asked the necessary questions that existed in formal questionnaires from parent and collected some information about parent kinship relationship, location, ethnicity and disease condition in their other children.

The sent samples to the laboratory were qualitatively surveyed by Guthrie test and considered the phenylalanine concentration less than 3.9 mg/dl as normal range and the values more than 3.9 mg/dl as non-normal range. In some cases that the test result was positive (test repetition twice), there were taken, for completion and confirmation of previous result, some samples from babies after spending several days without dietary restriction and sent to the laboratory for testing with HPLC method. At this stage and after final confirmation, those samples that had positive result were sent to the Pasteur Institute of Iran in order to differentiation of malignancy in babies and there were evaluated the level of neopterin, biopterin, phenylalanine and some other factors with HPLC and photometry methods in serum and urine samples.

RESULTS

Descriptive analysis shows that: There were recorded during 2year (2013-2014) screening program, the number of 50603 children (24746 babies in 2013 and 25857 babies 2014) in Ardabil province. Their segregation was as 52.5% boys (12990 boys) and 47.5% girls (11777 girls) in 2013, 52.24% boys (13507 boys) and 47.76% girls (12350 girls) in 2014.

The time of screening test, the 16cases increasing phenylalanine were declared positive by screening in phenylketonuria screening test in Ardabil province and all these people had plasma phenylalanine concentration higher than 6 mg/dl (363 micromoles per liter). Among 16 patients, 10 patients had the phenylalanine level over than 20 mg/dl (classic phenylketonuria) and the rest had the concentration about 6-20 mg/dl (mild hyper-phenylalanine), so this means that 62.5% of patients were stricken with classic phenylketonuria and 37.5% of them stricken with mild phenylketonuria (fig. 1).

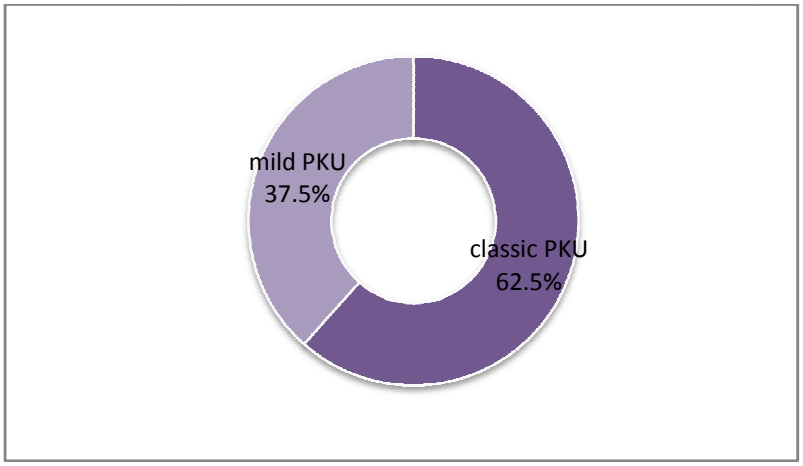

Fig. 1- percentage distribution of kinds of phenylketonuria of Ardabil province born babies at 2013 and 2014

According to the obtained results the phenylketonuria prevalence level in Ardabil babies recorded at screening equaled to 1 patient in 3163 births through 2 years.
In the present study 44% of patients are girls (7 cases) and 56% boys (9 cases).

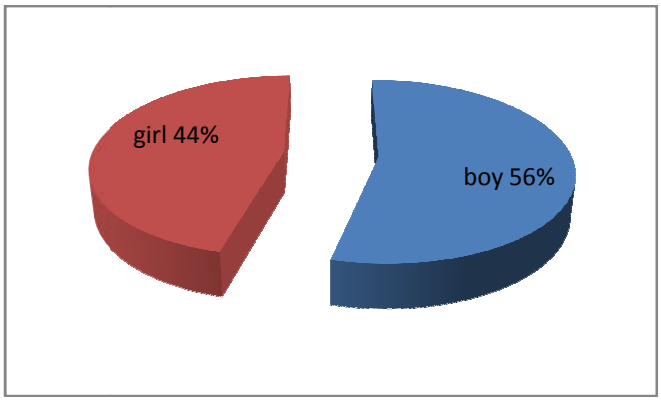

Fig. 2- gender distribution of patient born babies (PKU) in Ardabil province at the time of study

The patients' parent were considered in terms of being relative and determined that only 3 cases of marriages were non-intermarriage (19%) and other 13 cases (81%) were intermarriage (fig. 4).

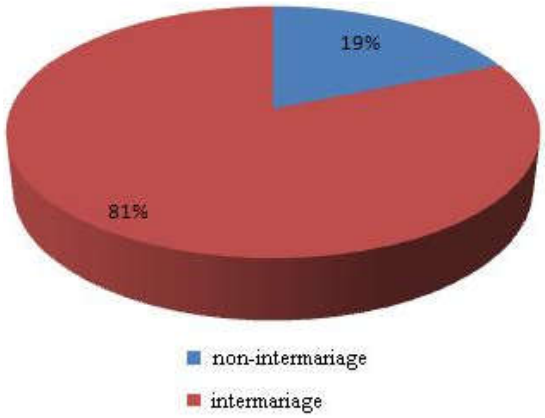

Fig. 3- percentage distribution of parent relativeness of babies stricken with phenylketonuria in Ardabil province at 2013-2014

Relativeness among parent in 7 cases was cousin, in 4 cases cousin-german and in 2 cases of far relatives. Patients were considered from lodging and literacy of parent; they were classified in the two group urbanite (11 patients) and rural (5 patients) for their lodging that the difference between these two groups was significant. The reason of this consideration was ascribing of lodging and awareness of parent from metabolic diseases and their relation with relative relationships which a significant relationship was also observed in this case (X^2 = 24.4, P_{value} = 0.0001). It was seen two cases suspicious for malignancy of lack of co-factor tetrahydrobiopterin among these 16 patients.

DISCUSSION

The screening program for phenylketonuria has been seriously and with national coverage started from 2013 in Ardabil province and each year by diagnosing of some people who were born with phenylketonuria defects has been able to highlight the role of screening in preventing of phenylketonuria side effects progress and curing patients. It was tried for the first time in Ardabil Health Network for collecting of available data and so the present study provided. There were taken the blood samples from the heel of all born babies in the first 72 hours after their birth and diagnosed phenylketonuria initial positive in them by Guthrie test. Then, there were done duplicate and supplementary tests for confirmation in positive cases and referred to Pasteur Institute of Iran for rejecting of malignancy cases of suspicious samples.

In this study the phenylketonuria prevalence was estimated 1:3163 in Ardabil province.With due attention to the obtained statistics from previous studies in Iran, the phenylketonuria prevalence is variable among 1:3000 to 1:60000 at different regions. The phenylketonuria prevalence has been obtained from different provinces of Iran that can hint to Shiraz [17] as 1:10000, Shiraz [18] as 1:4698 and Mashhad [19] as 1:17336. In the case of present study, with due attention to newly starting of screening the obtained ratio is promising and satisfactory and it shows that screening has been able to identify a high ratio of patients; it can be hinted from other point of view that this high ratio of phenylketonuria which is a kind of inherited metabolic disorder is indicative of high ratio of intermarriage between classes of Ardabil province. It can be also concluded from this statistic that the genetic counseling in this part of country is very essential for identifying carriers and heterozygous individuals in terms of common genetic disorders especially phenylketonuria.

It has been obtained the intermarriage prevalence among phenylketonuria patients about 81% in the present study that it is partly higher than the other available statistics in country; for example, it equals in Mashhad study [19] to 80%, in Mazandaran [20] to 60% and [21] to 82%, in Shiraz [18] to 71% and in Gilan [11] to 71.5%.

The phenylketonuria prevalence in this study with a low percentage was more in boys (56%) than girls (44%) (P value<0.08) that this result was supporter of the previous study in Iran which in Mashhad the percentof stricken boys was 51.3% and in Shiraz 70% [17, 19]. There are two reasons for this higher statistic among boys; the first and more important reason is that phenylketonuria is a kind of autosomal recessive disease that has significantly higher incidence among boys and second the combination of birthdays is in favor of boys at different provinces and the number of born boys is moreand this also causes that the involved people have more percentage of boys than girls.

The classic phenylketonuria prevalence (61.5%) was obtained more than the mild (38.5%) in this study that it means the number of babies that their phenylalanine level is higher than 20 mg/dl are more than those babies that their phenylalanine is less than 20 mg/dl. This result is in line with the results of other countries like US which there have been identified 85.1% of babies with plasma phenylalanine concentration over than 20 mg/dl and only 14.9% of them have phenylalanine less than 20 mg/dl. The present results are against of the obtained results in Iran that show 86.7% of patients have phenylalanine less than 20 mg/dl and only 13.3% of them have plasma phenylalanine concentration over than 20 mg/dl [18].

The number of identified patients' habitant in urban regions (Ardabil and Meshkinshahr) obtained as 69% and in rural regions as 31% in this study. This result is in line with the obtained results by Badiee *et al* [19] that reported 57% of patients were habitant in Mashhad and 43% in other cities. There are two reasons for this statistics; one of them is that population density is more in centers of provinces that this issue has been also seen in Mashhad study, but the classification of the present study has been city and village that it is different from classification type of Badiee et al [19]. Second, due to remoteness and inappropriate transportation conditions from village to Ardabil city there are referred less number of rural babies for childbirth to the hospital.

CONCLUSION

With due attention to the obtained results from the present study can conclude some general results as:

1- It seems necessary to use the available and supplementary methods like HPLC in centers for screening and monitoring of inherited disorders due to phenylketonuria high prevalence among Ardabil province Births.
2- It is necessary to do genetic counseling and screening in all marriages especially intermarriages due to high statistic of intermarriages among phenylketonuria patients.

With due attention to this issue that lodging and parent literacy obtained significant in preventing of phenylketonuria, thus it can be assumed that it is possible to decrease the prevalence of stricken with such disorders by increasing of parent literacy especially adults in relation to genetic and hereditary diseases. Therefore, having such centers especially in villages is necessary for increasing people awareness.

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