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Sibtain Ahmed
Aga Khan University, sibtain.ahmed@aku.edu

Hafsa Majid
Aga Khan University, hafsa.majid@aku.edu

Lena Jafri
Aga Khan University, lena.jafri@aku.edu

Aysha Habib Khan
Aga Khan University, aysha.habib@aku.edu

Zeba Zulfiqar Ali
Aga Khan University, zeba.zulfiqarali@aku.edu

See next page for additional authors

Authors

Sibtain Ahmed, Hafsa Majid, Lena Jafri, Aysha Habib Khan, Zeba Zulfiqar Ali, and Bushra Afroze

RESEARCH ARTICLE

Retrospective Study of Patients with Hyperphenylalaninemia- Experience from a Tertiary Care Center in Pakistan

Sibtain Ahmed[1], Hafsa Majid[2], Lena Jafri[3], Aysha Habib Khan[4], Zeba Zulfiqar Ali[5], Bushra Afroze[6]

Abstract

Objective: To assess the clinical and biochemical features as well as outcome of hyperphenylalaninemia patients.

Methods: The descriptive retrospective study was conducted at the Aga Khan University Hospital, Karachi, and comprised data from January 2013 to February 2017 of plasma amino acid analysed at the Biochemical Genetic Laboratory of patients with phenylalanine levels >120 umol/L. Medical charts of patients registered with the Metabolic Clinics were reviewed, while outside referrals were contacted by telephone to collect data on a pre-structured questionnaire. Data was analysed using SPSS 21.

Results: Of the 18 patients, 13(72%) were males. Overall median age was 606 days (interquartile range: 761) and median phenylalanine levels were 1280 (interquartile range: 935) umol/L. Phenylalanine hydroxylase deficiency was present in 5(28%) patients while 3(16.6%) had defects in the metabolism or regeneration of tetrahydrobiopterin. The most common clinical features was intellectual deficit and seizures 14(78%) each, followed by lighter hair colour 10(55.5%) and hypotonia 11(61%). High treatment cost was the leading reason for cessation of therapy in 7(39%) followed by refusal by patient's family 5(28%).

Conclusion: Most hyperphenylalaninemia cases were diagnosed late when intellectual disability had already developed.

Keywords: Phenylalanine, Hyperphenylalaninemia, Classification, Phenylalanine hydroxylase deficiency, Tetrahydrobiopterin defects. (JPMA 69: 509; 2019)

Introduction

Disorders of phenylalanine (Phe) metabolism are one of the most frequently presenting inherited metabolic disorders (IMDs).[1] They are a heterogeneous group of autosomal recessive disorders due to deficiency of the enzyme, phenylalanine-4-hydroxylase (PAH) or defects in the metabolism or regeneration of its cofactor tetrahydrobiopterin (BH4).[2] The BH4 is synthesised from guanosine triphosphate cyclohydrolase (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), sepiapterin reductase (SR) and is regenerated by dihydropteridine reductase (DHPR) and pterin carbinolamine-4-dehydratase (PCD).[3] Neonates with PAH deficiency are usually asymptomatic at birth except a tendency towards lower birth weight and smaller head circumference, but,

if left untreated, can present with growth failure, poor skin pigmentation, microcephaly, seizures, global developmental delay (DD), severe intellectual deficit (ID) and movement disorder. Clinical features of BH4 defects include ID, convulsions, disturbance of tone and posture, drowsiness, irritability, abnormal movements, recurrent hyperthermia, hyper-salivation and swallowing difficulties,[4] however, GTPCH, PTPS, DHPR and PCD defects have their peculiar biochemical profile. Hyperphenylalaninemia (HPA) is identified through quantification of plasma amino acid (PAA). Further evaluation of HPA occurring as a secondary consequence of a defect in the metabolism or regeneration of BH4 is done through analysis of urine or blood neopterin, biopterin and measurement of DHPR activity.[5,6] Differentiation between PAH deficiency and defect in metabolism or regeneration of BH4 is important for the initiation of disease-specific treatment, for better

[1-4] Department of Pathology and Laboratory Medicine, Aga Khan University,
[5,6] Department of Paediatrics and Child Health, Aga Khan University, Karachi.
Correspondence: Bushra Afroze. e-mail: bushra.afroze@aku.edu

outcomes, carrier screening for high-risk family members, proper genetic counselling and future reproductive options for the carriers. Patients with PAH deficiency require Phe-restricted diet. On the contrary, for BH4 defects, Phe-restricted diet is often not needed but early supplementation with dopamine and serotonin precursors, and synthetic BH4 is required to attain symptomatic improvement.[7]

The prevalence of HPA due to either deficiency of PAH enzyme or defects in the metabolism or regeneration of BH4 is reported to be 1:10,000.[8] However, the prevalence of BH4 deficiency is rare, accounting for 2-3% of HPA cases.[9] The true incidence of HPA in Pakistani population is unknown. A study reported five cases of HPA 2013-14.[10] Data on the aetiological causes of HPA is lacking in Pakistan. The current study was planned to assess the clinical, biochemical features, outcome and aetiology of patients with HPA.

Material and Methods

The descriptive retrospective study was conducted at the Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, in collaboration with the Department of Paediatrics and Child Health, Aga Khan University Hospital (AKUH), Karachi, and comprised data from January 2013 to February 2017.

Data was related to patients who reported with plasma Phe levels >120umol/l on PAA.

In the first phase, reports of patients with elevated Phe>120 umol/L were reviewed by a chemical pathologist and a metabolic physician. PAA patterns consistent with liver disease were excluded. The characteristic patterns of liver diseases on PAA was either elevated Phe, tyrosine, ornithine, methionine and decreased branched chain amino acids (leucine, valine, and isoleucine) or elevated Phe, tyrosine, methionine and histidine.[11] Dissimilarity exists among different centres on the methodology used and age of screening for HPA. Also, blood Phe levels, taken as positive result requiring further investigations, varies from 120umol/L to 240umol/L.[12,13] The cut-off >120 umol/L is used for newborn screening (NBS) done via Tandem Mass Spectrometry (TMS), but, as we performed PAA quantification using Biochrom based on high performance liquid chromatography (HPLC), which is not a method for NBS, we used the higher cut-off of Phe i.e. 240 umol/L for HPA. In the second phase, medical charts of patients registered at metabolic clinics were reviewed,

while outside referrals were contacted by telephone to collect information about further laboratory tests (urine or blood neopterin, biopterin, DHPR activity or molecular tests) and treatment received. This was done on a pre-structured questionnaire.

For PAA analysis, 3-4ml of blood samples was collected in lithium heparin tube. Samples were transported in dry ice to the Biochemical Genetic Laboratory (BGL) after centrifugation from outreach phlebotomy centres. All samples were stored at -20°C prior to analysis. A questionnaire focussing on clinical and biochemical information was filled for the in-patients and for patients whose samples were received at the phlebotomy centres of AKUH.

The quantification was performed by cation-exchange HPLC (Biochrom 30+ model 440/570 nm), with a lithium column of 4.6mm diameter. Standard, control and specimens were deproteinised with sulfosalicylic acid 10% and norleucine was added as internal standard. The results were analysed by EZ chrome software 3.31. Quality control, validation and proficiency testing validation for amino acids were accomplished according to Clinical and Laboratory Standards Institution (CLSI) guidelines.[14] Two levels of commercially available controls were run with each batch of 10 samples.

Study was commenced after approval was obtained from the institutional ethics committee.

Data was analysed using SPSS 21. Frequencies and percentages were calculated for gender, consanguinity, clinical presentations, biochemical features and aetiological classification of HPA. Data for age and blood Phe levels was skewed and, hence, median and interquartile ranges (IQR) were calculated.

Results

Over the period under review, 3057 patients were tested for PAA. Of them, 34(1.1%) had Phe levels >120 umol/l and comprised the initial sample. Subsequently, 12(35%) patients showing PAA pattern consistent with liver disease and 4)11%) with missing data were excluded. The final sample had 18(53%) patients (Figure 1). Of them, 4 (22%) patients had expired.

There were 13(72%) males and 5(28%) females. Median age of the males and females were 547 days (IQR: 592) and 665 days (IQR: 2491) respectively. Overall median Phe level was 1280 (IQR: 935) umol/L. Parental consanguinity was observed in 17(94.4%) patients.
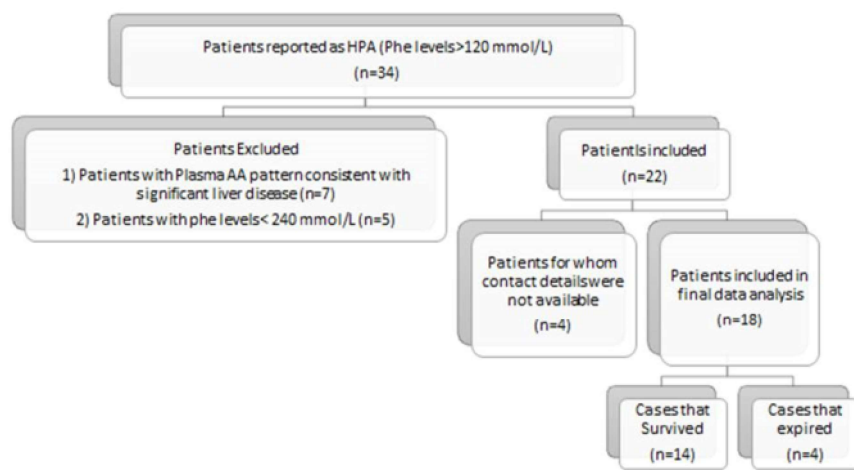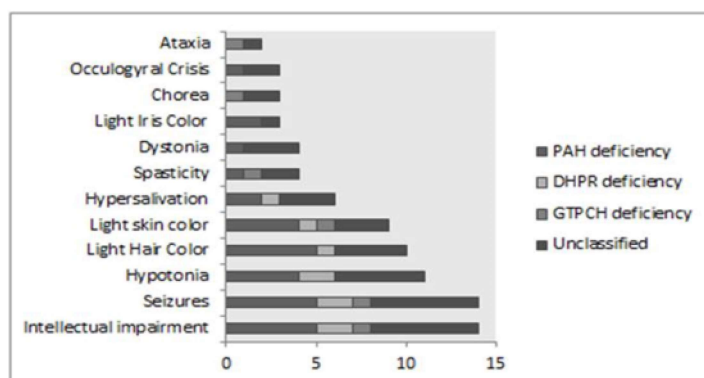
Figure-1: Flowchart showing frequency and outcome of patients with hyperphenylalaninemia (n=34).

*Defects in metabolism/regeneration of tetrahydrobiopterin include DHPR and GTCH deficiency.

Figure-2: Clinical features of patients with phenylalanine hydroxylase enzyme deficiency or defects in metabolism / regeneration of tetrahydrobioptern (n=18).
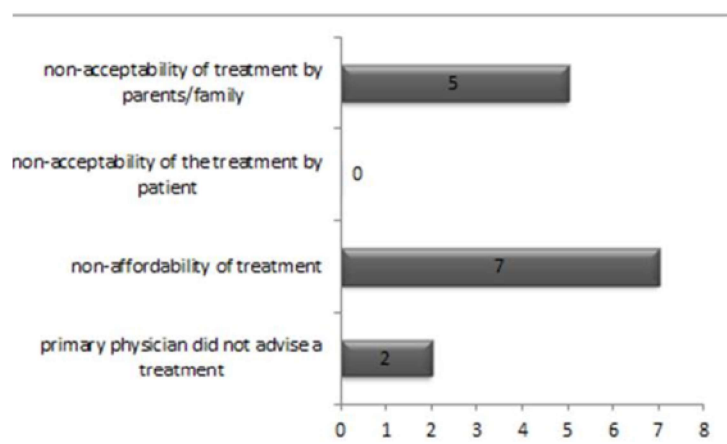

Figure-3: Reasons for cessation of therapy in patients with Phenylalanine hydroxylase enzyme deficiency or defects in metabolism/regeneration of tetrahydrobiopterin (n=14).

Further diagnostic testing, including blood pterin levels and DHPR activity, was done in 8(44%) cases while the rest were not advised further workup by their primary physicians. The most common clinical features recorded were ID 14(78%) and seizures 14(78%), followed by hypotonia 11(61%) and lighter hair colour 10(56%) Figure 2). No significant correlation was found between a particular presenting feature and disease type (p>0.05).

There were 5(28%) patients with PAH deficiency who were started on a Phe-restricted natural diet, Phe-omitted amino acids mixture and symptomatic treatment comprising anti-convulsants and muscle relaxants. In 1(20%) patient, treatment was discontinued after 4-5 months due to non-acceptability of treatment by the family.

L-dopa, 5-hydroxy (OH) tryptophan and folinic acid and symptomatic treatment of anti-convulsants and muscle relaxants was advised to 2(11%) patients with DHPR deficiency. However 1(50%) patient failed to continue treatment after 1.5 years due to unavailability of medications.

There were 2(11%) patients with GTPCH deficiency who were started on treatment with L-dopa, 5-OH tryptophan and BH4 and symptomatic treatment of anti-convulsants and muscle relaxants, which was discontinued by parents after 1 year due to unaffordability.

Patients with HPA who were not further evaluated were categorised as unclassified HPA 10(55.5%). In this group, 5(50%) patients were advised Phe-restricted diet based on low protein diet with no meat, egg and milk, which was followed only by 2(40%) patients. However, no treatment was advised by the physicians in 5(50%) cases. The high cost of treatment was the leading reason for cessation of therapy 7(38%) patients, followed by non-acceptability by patient's family 5(28%) (Figure 3).

Discussion

Patients with HPA must be diagnosed and treated before manifestation of clinical symptoms to prevent ID. Asia-Pacific countries, including Pakistan, Bangladesh and

India, with large numbers of births lack a comprehensive NBS programme for inherited metabolic disorders (IMDs).[15,16] The older age of presentation of most patients in this study points to the fact these patients were screened for IMDs when they already had irreversible ID. The prevalence of HPA is higher in Turks (1 in 2600 live births) and Saudi Arabia (1 in 6000) due to consanguinity compared to Caucasian countries like Finland where occurrence is as low as 1 in 200000 births.[5,17,18] Most physicians in Pakistan usually do not evaluate patients with ID for IMDs. Patients are often evaluated for IMD in clinical presentation of 'metabolic crisis' or 'neuro-regression'. Whereas, patients with HPA neither present with 'metabolic crisis' nor 'neuro-regression' but the classic presentation of HPA is ID, seizures and movement disorders. Thus diagnosis of HPA is missed in Pakistan and could be a reason of less number of cases compared to the Turks and Saudis with similar rates of consanguinity. The importance of screening individuals with ID for HPA is demonstrated in an Iranian study undertaken in institutionalised individuals, where the prevalence of HPA was fund to be 4%.[19]

Literature search shows that PAH deficiency is the most common HPA encountered compared to the prevalence of BH4 deficiency, which constitutes about 3% of HPA cases.[20] In our study, PAH deficiency was the most common cause of HPA.

Urine or blood pterins and DHPR activity was done in only 8 cases and all were followed up by a metabolic physician. This could be due to the local non-availability of the pterins analysis and DHPR activity needed for the aetiological work-up of HPA in Pakistan, logistic limitations in outsourcing of these tests to overseas labs, and lack of awareness of the general paediatricians about the diagnostic approach for HPA. Classification of HPA is essential because the prognosis, treatment and outcome of BH4 defects and PAH deficiency is different from each other. Not all HPAs need dietary treatment which is age and gender-specific, like Phe-restricted diet is required in PAH deficiency but is not needed in BH4 defects, in which dopamine and serotonin precursors and synthetic BH4 is needed.[21,22]

Majority of the patients either did not start treatment or discontinued Phe-restricted diet mainly due to financial constraints and non-acceptability of the food for special medical purpose (FSMP) by the families who did not accept the concept of diet modification as a treatment.

Lack of knowledge of patient families about the consequences of the disorder and potential benefits of treatment, superimposed by high cost, are reported as a common factor leading to noncompliance to treatment regimens.[23,24] An estimated average monthly treatment cost for a patient needing FSMP in Pakistan ranges from €400-€1000, owing to the high tax imposed by the government on its import. This often creates a dilemma for families to sustain a life-long treatment.

There were certain limitations to our study. Firstly, most patients were referred to the centre for diagnostic evaluation because of a suspected IMD in clinical presentation of 'metabolic crisis' or 'neuro-regression'. Therefore, the results may not be generalised to the community or non-referred samples. Secondly, the sample size was small, limiting the power of the study and there is a likelihood that significant correlation between a particular presenting feature and disease type may arise with larger sample size.

Conclusion

There is need for local availability of pterin analysis and DHPR activity for easy access to physicians who can follow the appropriate diagnostic approach for patients with HPA. The outcome of patients with HPA due to PAH deficiency can be improved significantly with early detection through NBS allowing early intervention, which has to be sustainable.

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