THE PREVALENCE OF PHENYLKETONURIA AMONG CHILDREN WITH MENTAL RETARDATION IN KELANTAN

by

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Dissertation Submitted In Partial Fulfilment Of The Requirements For The Degree Of Master Of Medicine (Chemical Pathology)

UNIVERSITI SAINS MALAYSIA
Nov. 2001

ACKNOWLEDGEMENT

I would like to thank my supervisor, Dr Rowani Mohd Rawi from the Department of Paediatrics, for her excellent guidance and continuous support in carrying out the study and in writing up this dissertation.

My greatest thanks to my husband Dr Mohamed Rusli Abdullah for his excellent guidance through out the preparation of this dissertation and also for being very supportive and understanding.

My greatest thanks to Associate Professor (Dr) Nadiger, Department of Chemical Pathology, for his contribution, advice and guidance in the preparation of this dissertation.

Special thanks to the IEM team, Encik Saruddin Abbas, Encik Zafuan Zahari and not forgetting Cik Raihan whom made it possible in carrying out the study.

My greatest thanks to Puan Rosliza, for her excellent guidance, cooperation and time in conducting the test at the Microbiology laboratory.

I would also like to thank Dr Md Radzi Johari, Head of Department of Microbiology for allowing me to use the facilities available in his department.

To my beloved children; Zaquan, Syamim, Syauqin and Afnan, thank you for being patience and supportive.

I would like to thank Universiti Sains Malaysia for the short term grant without which this study would not have been possible.

Last but not least, I would like to thank all who took part in this study, especially to all the Schools, Community and Clinic-based Rehabilitation Centres and individuals whom I have not listed here.

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LIST OF ABBREVIATIONS

1. IEM	Inborn Errors of Metabolism
2. PKU	Phenylketonuria
3. MOH	Ministry of Health
4. SWD	Social and Welfare Department
5. ED	Education Department
6. IQ	Intelligent Quotient
7. HPLC	High Performance Liquid Chromatography
8. BH_4	Tetrahydrobiopterin
9. PAH	*Phenylalanine hydroxylase*
10. DNA	Double-stranded nucleic acid
11. BIA	Bacteria Inhibition Assay
12. MSUD	Maple Syrup Urine Disease
13. HCU	Homocystinuria
14. HE	Histedinemia
15. TMS	Tandem Mass Spectrometry
16. NaOH	Sodium Hydroxide
17. QCT	Quality Control Test
18. ATCC	American Type Culture Collection
19. CCRC	Community- and Clinic-based Rehabilitation Centre
20. "lembam" and LC	Lembam and last classes
21. S&S	Schleicher and Schull
23. DBS	Dried Blood Spot

adalah secara signifikannya lebih rendah dikalangan responden lembam dan KA dan PPKK berbanding dengan dua lagi kumpulan. Pada masa yang sama didapati tiada perbezaan signifikan pendapatan responden lembam dan KA dan PPKK, dan diantara responden normal dan terencat akal di sekolah. Ujian phenylalanine adalah negatif bagi 1170 responden yang diambil spot darah mereka.

Walaupun keputusannya adalah negatif, responden kajian hanyalah 5% daripada unjuran penduduk mengalami terencat akal, yang tidak terlibat didalam kajian ini kerana mereka tidak menghadiri institusi-institusi yang di lawati. Teknik makmal yang digunakan adalah tepat, dan ralat telah dikenalpasti secara sistematik dan dicegah untuk memastikan keputusan adalah sah dan boleh dipercayai. Berdasarkan kepada keadaan dan kesudahan penyakit, PKU adalah masalah yang penting kepada sector kesihatan dan social, dan dengan itu memenuhi criteria untuk diadakan satu program saringan. Walaubagaimanapun, keputusan negatif yang diperolehi oleh kajian ini memerlukan penyiasatan lanjut dilakukan dalam masa yang terdekat ini untuk meyakinkan pihak berkuasa akan perlunya program ini walaupun agak mahal. Kajian ini perlu di panjangkan untuk melibatkan pesakit lain dan juga pesakit daripada negeri-negeri lain. Kemungkinan juga, adalah perlu untuk mengkaji cara-cara lain bagi menyaring PKU.

ABSTRACT

THE PREVALENCE OF PHENYLKETONURIA AMONG CHILDREN WITH MENTAL RETARDATION IN KELANTAN

The prevalence of phenylketonuria (PKU) in Malaysia to date is not known since no study has been conducted to address the subject. The objectives of this study were to determine the prevalence of PKU among the mentally retarded children in Kelantan, to determine the feasibility of carrying out a screening programme for PKU in newborns and to establish a method for PKU screening should the need arise later on. The study was a cross-sectional survey involving all the schools with special programmes for mentally retarded children and community- and clinic-based rehabilitation centers (CCRC) in Kelantan. All children listed and attended these two institutions were taken as the study samples. A validated questionnaire was used to obtain personal and socioeconomic information of the patients and their families. The questionnaire was collected together with the written consent for the study before the final list of respondents was recorded. Blood spots on filter papers were collected by a finger prick method using automated disposable lancet. Validation of the information in the questionnaire, physical examinations and blood spots collection were done at the respective schools and CCRC. Phenylalanine assays were done using the Bacteria Inhibition Assay (BIA), also known as the Guthrie method.

The BIA method was optimized in local laboratory before it is used to test the study samples. A total of 24 schools and 34 CCRC were visited during the study period all over Kelantan. Out of 1715 respondents who returned the questionnaire and consented to take part in the study, only 1568 agreed to have blood spots taken and were present during the blood collections. Other then children with mental retardation, samples were also taken from normal and "lembam and last classes (lembam and LC) children. Out of the 1319 school-based respondents, 239 (18.1%) were mentally retarded and 276 (20.9%) were lembam and LC. All the 396 repondents from CCRC were mentally retarded children. The proportion of boys was significantly higher in school-based mentally retarded, lembam and LC and CCRC respondents, but lower in the normal group. The majority of

the respondents were malays, conforming to the population structure of Kelantan. Household income was significantly lower in the lembam and LC and CCRC respondents compared to the other two groups. Likewise, there was no significant difference in income between the lembam and LC and CCRC respondents, and between the normal and school-based mentally retarded respondents. The phenylalanine assays were negative for the 1170 respondents who had their blood spots taken.

Although the result was negative, the study respondents constituted of only 5% of the projected population with mental retardation, which were not captured by this study because they did not attend any of the institutions visited. The laboratory technique used was accurate and errors had been systematically addressed and prevented to ensure the results were valid and reliable. Phenylketonuria, given the nature and outcome of the disease, is a considerable problem to both the health and social sectors, and fits the criteria to have a screening programme put in place. However, the negative result of this study requires further investigations to be conducted in the near future to convince the authority as to the need for such an expensive programme. This study should be extended to include patients not covered here and also patients in other states. Perhaps, it will also be beneficial to examine other methods of screening for PKU.

INTRODUCTION

CHAPTER 1. INTRODUCTION

1.1 MENTAL RETARDATION

1.1.1 DEFINITION

Mental retardation is subaverage intellectual ability present from birth or early infancy. It is the most common lifelong handicap in developed nations and it is likely to consume more professional and financial resources than any other disabling conditions (Munro *et al.* 1986). Mental retardation has traditionally been defined in terms of a threshold score on measurement of intelligence. An individual with an Intelligence Quotient (I.Q) score below a specified score would be classified as mentally retarded. However, the definition has recently been expanded to include social adaptation.

Based on Diagnostic and Statistical Manual IV of American Psychological Association, mental retardation means significantly subaverage general intellectual functioning existing concurrently with deficits in adaptive behaviour and manifested during developmental period, that adversely affects a child's educational performance (National Information Centre for Children and Youth With Disabilities, 2001). Deficits or impairment in adaptive behaviour must be in at least two of the following areas; communication, self-care, home living, social or interpersonal skills, use of community resources, self-directed functional academic skills, work, leisure health and safety. The onset of mental retardation should be

before the age of 18 years. Mental retardation was originally classified into four levels based on the IQ scores (Table 1.1). It was later reclassified to just two levels, mild and severe. However, this new definition is still controversial (Batshaw, 1993) and has not been fully adopted.

Table 1.1. The levels of mental retardation based on IQ scores.

Level of Retardation	IQ Scores	Characteristics
Mild	50 – 70	A majority of all the mentally retarded. Usually show no physical symptoms of abnormality. Individual with higher IQ's in this category can marry, maintain a family, and work in unskilled occupations. Abstract reasoning is difficult for those with the lower IQ's in this category. Capable of some academic learning.
Moderate	35 -49	Often lack physical coordination. Can be trained to take care of themselves and acquire some reading and writing skills. Abilities of a 4-7-year old. Capable of living outside an institution with their families.
Severe	20 - 34	Only a few can benefit from schooling. Can communicate vocally after extensive training. Most require constant supervision.
Profound	< 20	Mental age is < 3 years old. Very limited communication. Require constant supervision. Can learn to walk, utter a few simple phrases, and feed themselves.

Source: The Association of Retarded Citizen of the United States (2001).

1.1.2 PREVALENCE OF MENTAL RETARDATION

Mental retardation is not a disease. Children with mental retardation become adults and they do learn but, slowly and with difficulty. However, for some of them, even with proper supervision they ended with limited achievement (The Association of Retarded Citizen of the United States, 2001). Various studies have been conducted in communities to determine the prevalence of mental retardation and concluded that 3% of general population has mental retardation (Munro, 1986).

Based on the 1990 census, an estimated 6.2 to 7.5 million people have mental retardation in the United States of America (The Association of Retarded Citizen of the United States, 2001) of which, more than 85% fall into the mild range. The ratio of males to females is 1.6:1 in mild retardation, but is close to unity in severe mental retardation. Another interesting finding is the existence of different socio-economic status in different levels of mental retardation. The prevalence of mild mental retardation was found to be higher in lower socio-economic classes whereas, no class bias exists in severe retardation (Batshaw, 1993).

Mild mental retardation is usually an isolated disability whereas severe retardation is often accompanied by associated deficits that further limit the child's adaptive abilities and affect outcome. The prevalence of these associated deficits roughly correlates with the severity of mental retardation and include cerebral palsy, visual deficits, seizure disorders,

communication deficits, feeding problems, psychiatric or pervasive developmental disorders and attention-deficit hyperactive disorder.

In Malaysia, the actual prevalence of mental retardation had not been reported anywhere. Currently, there is three governmental bodies that are involved with mentally retarded people: the Ministry of Health (MOH), Social and Welfare (SWD) and Education (ED) Departments. Based on the registration list from SWD, an estimation of 28 129 people are mentally retarded in the year 2000 (Rashid, 2000). This estimation comprised of both children and adults but the exact age distributions and gender are not known.

In the education sector, special education for the special children was first introduced in 1920 with the opening of St. Nicholas Primary School in Melaka (Abdullah, 2000). However, it was not until the Cabinet Report 1979, comprehensive initiatives to cater for the education needs of special children really took off. Based on the report, starting from 1981, the MOH and Ministry of Unity and Welfare were instructed to care and provide services and training for the children with special needs. The Government of Malaysia has also given her commitments in implementing recommendations agreed in international declarations such as, The United Convention On the Rights of The Child (1989), Education for All (1990), Standard Rules on the Equalisation of Opportunities for Person With Disabilities (1993) and The Salamanca Statement (1994). Up to the year 2000, there are approximately 7,223 children with learning disabilities including mentally retarded children, attending schools all over Malaysia (Abdullah, 2000)

1.1.3 THE AETIOLOGY OF MENTAL RETARDATION

Mental retardation can be caused by any condition that impairs development of the brain before or during birth, or in the childhood years. Hundreds of causes have been discovered, but most children with mild mental retardation do not carry an etiologic diagnosis. In children with severe retardation, however, there is a much greater chance of making a diagnosis. The most common identifiable causes of severe retardation in Western countries are the congenital group of disorders that include chromosomal disorders and prenatal toxins. In Malaysia, perinatal problems were considered to be one of the commonest causes although no specific data were available.

In developed countries, the most common diagnoses are fragile X syndrome, Down syndrome and fetal alcohol syndrome which, together account for about one third of all identifiable cases of mental retardation (Batshaw, 1993). For the other two thirds, diagnostic studies are done based on historical information and physical findings. A child with an unusual appearance or multiple anomalies should be referred to a genetic center for consultation. On the other hand, a child with loss of milestones may have an inborn error of metabolism, which requires metabolic screening.

Reber (1992) suggested that it is essential to pursue an etiologic diagnosis in cases of mental retardation in order to:

1. identify treatable medical conditions

2. provide adequate explanation to families regarding the disorder to help them cope with the feelings of anger, guilt and blame
3. provide databases that will determine the direction of the country's health, educational and social services
4. identify all associated handicapping features of the individual's disorder.

The causes of mental retardation can be divided into several categories (The Association of Retarded Children of the United States, 2001):

a. Genetic conditions

Mental retardation results from abnormality of genes inherited from parents, or disorders of the genes that occur during pregnancy caused by infections, over-exposure to radiations and other factors. Inborn errors of metabolism such as Phenylketonuria (PKU), fall into this category and shall be dealt with in detail later part of this introduction.

b. Problems during pregnancy

Use of alcohol or drugs by pregnant mothers can cause mental retardation. Malnutrition, rubella infection, glandular disorders, diabetes and many other illnesses of the mother during pregnancy can result in a child being born with

mental retardation. Physical malformations of the brain and HIV infection originating in prenatal life may also result in mental retardation.

c. Problems at birth

Although any birth condition of unusual stress may injure the infant's brain, prematurity and low birth weight causes serious problems more often than any other conditions especially in developing countries.

d. Problems after birth

Childhood diseases such as whooping cough, chicken pox and measles which, can lead to meningitis and encephalitis, can damage the brain. Accidents such as a blow to the head or near drowning can also cause mental retardation.

e. Poverty and cultural deprivation

Children from poor families may become mentally retarded because of malnutrition, disease-producing conditions, inadequate medical care and environmental health. Children from disadvantaged areas may also be deprived of many common cultural and day-to-day exposures experienced by other youngsters.

Intensified and advance research have produced significant results and provided methods and means of preventing mental retardation by identifying and categorizing specific causes for the retardation. For example, 250 cases of PKU and 1000 cases of congenital hypothyroidism were prevented from becoming mentally retarded annually. This was achieved by effective newborn screenings and dietary and hormone replacement (Alexander, 1998).

1.2 INBORN ERRORS OF METABOLISM AND MENTAL RETARDATION

Over 300 human diseases due to IEM are recognized and the number is constantly increasing as new concepts and techniques are available for identifying biochemical phenotypes (Scriver, 1995). Many of these disorders remain undetected or misdiagnosed. Making a clinical diagnosis of IEM is difficult because individual inborn errors are relatively rare and many physicians failed to consider them in acute situation until more common conditions have been ruled out.

A large number of IEM give rise to neurological symptoms, developmental delay and mental retardation. Surveys of large and representative groups of mentally retarded people indicate that genetically determined metabolic disorders are the cause of 3-7% of severe mental retardation (Jakab, 1982). Diagnosis is complicated by the fact that there may be more than a thousand separate disorders that can cause mental retardation. At present, there are two major ways of diagnosing genetic disorders associated with mental retardation; by

mass screening programmes such as newborn screening programmes for treatable metabolic diseases like PKU and hypothyroidism, and a high index of clinical suspicion with expert laboratory services. Current advances in screening technology have allowed several hundreds of diagnoses to be made.

Most IEM are autosomal recessive mode of inheritance. Majority of the IEM are due to single mutation with all other processes function normally. This often resulted in the children having a normal physical appearance. Abnormal appearance in IEM usually develop postnatally comparative to chromosomal disorders, which are present at birth. For example, in mucopolysaccharidosis, there is a gradual postnatal accumulation of polysaccharide in skin, visceral organs and bones due to genetically determined deficiency of one specific enzyme. The abnormal facies manifested in the affected children is a progressive postnatal phenomenon related to the extent of polysaccharide accumulation. Meanwhile, progressive loss of mental and neurological functions becomes evident within the first few months after birth.

1.2.1 PREVALENCE OF IEM WITH MENTAL RETARDATION

There are many genetic metabolic disorders associated with mental retardation of which hypothyroidism and PKU are the two most common. The prevalence of some genetic metabolic disorders that associated with mental retardation are listed Table 1.2 (Scriver, 1995).

Some of the diseases are prevalent in specific ethnic groups, such as Gaucher disease and Tay-Sachs disease that are most prevalent in the Ashkenazi Jews than in general or other populations. To date, there are no available data in Malaysia regarding genetic metabolic diseases. There has also been no study done looking into IEM as the aetiology for mental retardation. Therefore, the prevalence in general Malaysian population and its relationship with specific ethnic groups are still obscured.

Table 1.2 Prevalence of genetic metabolic diseases associated with mental retardation.

Diseases	**Prevalence**
Congenital hypothyroidism	1:3 500
Phenylketonuria	1:10 000
Sanfilippo syndrome	1:24 000
Metachromatic leukodystrophy	1:100 000
Fabry's disease	1:40 000
Gaucher disease (Ashkenazi Jews)	1:2 500
Tay-Sachs disease (Ashkenazi Jews)	1:3 000
(General population)	1:300 000
Hunter syndrome	1:70 000
Galactosemia	1:35 000
Hurler syndrome	1:100 000
Maple syrup urine disease	1:180 000
Homocystinuria	1:40 000

1.3 PHENYLKETONURIA (PKU)

1.3.1 HISTORICAL PERSPECTIVES

Phenylketonuria (PKU) is an IEM. It is a rare but treatable genetic disorder. The first IEM was actually discovered in 1810 by Wallaston when he found a substance in the bladder stones of his patients. However, it was only in 1902 that this substance was identified chemically as an amino acid, and named cystein. Sir Archibald Garrod later grouped together diseases like alcaptonuria, albinism, pentosuria and cystinuria as IEM. Since then, the number of these inherited diseases recognized had risen, including PKU. Some of these IEM are treatable and it is usually necessary to recognize them in early infancy to prevent irreversible damage to various organs such as brain, liver, kidneys and eyes.

The incidence of PKU varies with countries and ethnic groups from rare, to relatively common. Though rare conditions would not normally attract much attention, PKU is an exception since it is a treatable genetic disease. Researchers have gone to great details in generating genetic knowledge and methods of treatment for this inborn error. PKU screening is already being carried out on virtually all newborns in every American State, Canada, Australia, New Zealand, Japan and many other countries throughout the world (Paul, 2000).

1.3.2 EPIDEMIOLOGICAL DESCRIPTIONS

The worldwide incidence of PKU is 1:6000 – 1:78000 live birth. Although PKU has traditionally been considered more prevalent among Caucasians with 1:10000 -15000 live birth (Therell, Jr., 1993), exceptions occur that may indicate ethnic composition is the predominant factor that influence incidence.

The incidence of classical PKU of 1:5300 has been reported in Scotland and Ireland, while the reported incidence in Canada was 1:22000 (Aldis *et al.* 1993). More variant exist among the Asian population, with incidence reported in Japan and China were 1:120000 and 1:20000 live birth, respectively (Aldis *et al.* 1993). However, the prevalence among mentally retarded children is higher. Studies conducted in Taiwan and Kuwait reported an incidence rate of 1:270 – 1:362 mentally retarded children, and 7 cases among 451 institutionalized mentally retarded persons, respectively (Teebi *et al.* 1987).

1.3.3 CLINICAL FEATURES

Phenylketonuria (PKU) carries serious clinical consequences to the affected neonates or young infants that include mild or severe mental retardation, physical handicap and even fatality. Ninety percent of the patients with severe mental retardation had an IQ of less than 50 (Paul, 2000). Features other than mental retardation include a 'mousy' odor, light pigmentation, peculiarities of gait, abnormal stance and sitting posture, eczema and

epilepsy. Cataracts and brain calcification are two manifestations frequently overlooked in untreated classic PKU. Fortunately, mental retardation can be prevented if newborns are placed on a special diet from which most of the phenylalanine has been removed. Early diagnosis for this disorder has proven very effective in treatment or management of the disease (Rashed *et al.* 1997).

1.3.4 BIOCHEMICAL FEATURES

In general, the defect in PKU is due to impaired ability to convert phenylalanine to tyrosine. This causes toxic accumulation of phenylalanine and its metabolites such as phenylpyruvate, leading to phenylketonuria and phenylacetate. The defect can be divided into 3 groups; defects in *Phenylalanine hydroxylase*, defiency in *Dihyropterine reductase* and disturbed de novo tetrahydrobiopterin biosynthesis

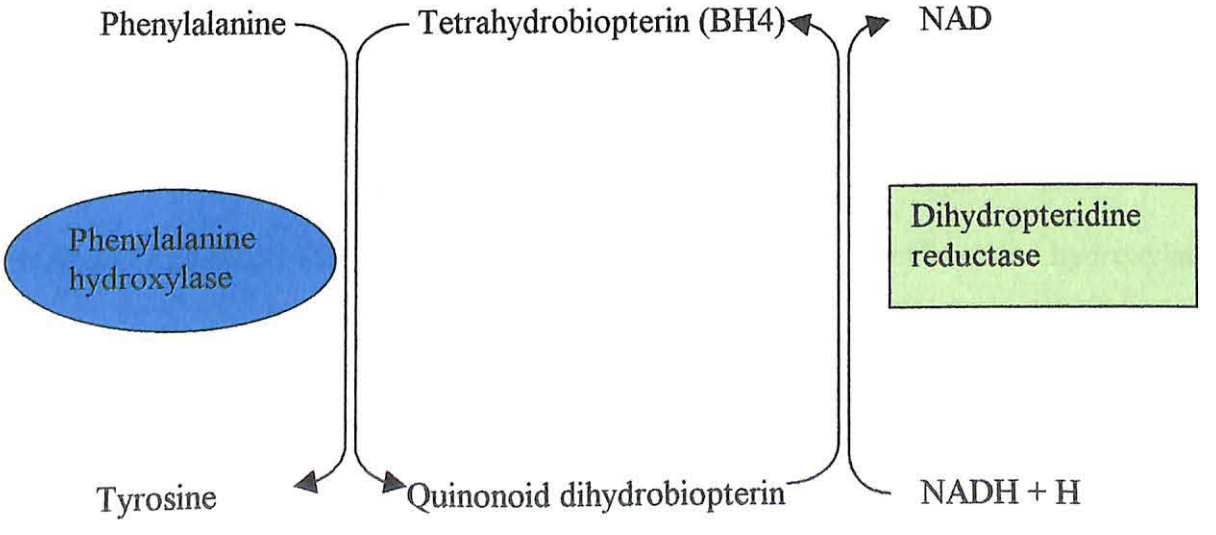

Figure 1.1. Metabolic pathway of phenylalanine

The commonest type of PKU associated with mental retardation is deficiency in the *Phenylalanine hydroxylase* causing hyperphenylalaninaemia, known as classical or typical PKU. Classical PKU is characterized by plasma phenylalanine concentration of more than 20 mg/dL or higher, on free diet with low or normal concentration of tyrosine. They have less than 1% normal activity of *Phenylalanine hydroxylase*. Now there are at least six mutant alleles, which have been recognized for the hydroxylase locus at chromosome 12q22 – q24.1, all of which are associated with the classical phenotype (Aldis *et al.* 1993).

Based on current laboratory and clinical findings, there are a lot of heterogeneity to *Phenylalanine dehydroxylase* disorders. It is now apparent that there are variant forms of abnormal phenylalanine metabolism, which do not result in mental retardation. These conditions are referred to as persistent benign hyperphenylalaninemias with plasma phenylalanine levels below 10 mg/dL on a normal diet with over 5% of normal enzyme activity (Guttler and Lou, 1990).

Other serious defect in phenylalanine metabolism involves the production of tetrahydrobiopterin (BH_4), the cofactor required for phenylalanine hydroxylation. Defective production of BH_4 or deficiency of enzyme dihydropteridine reductase is also associated with abnormal neurological development due to impaired synthesis of certain neurotransmitter precursor. They are referred to as malignant hyperphenylalaninemias and have poor prognosis compared to classical PKU.

1.3.4.1 Biochemical mechanism of PKU

When *Phenylalanine hydroxylase* is deficient, alternate pathways are used to metabolize phenylalanine (Figure 1.2). Phenylalanine is transaminated to phenylpyruvic acid, which is reduced to phenyllactic acid. Phenylalanine can also be decarboxylated to phenylacetic acid, which is then conjugated to phenylacetylglutamine. These derivatives are produced in excess and appear in the urine. However, the derivatives of phenylalanine are not found to be toxic in humans. Therefore, the probable cause for neurotoxicity is the phenylalanine itself.

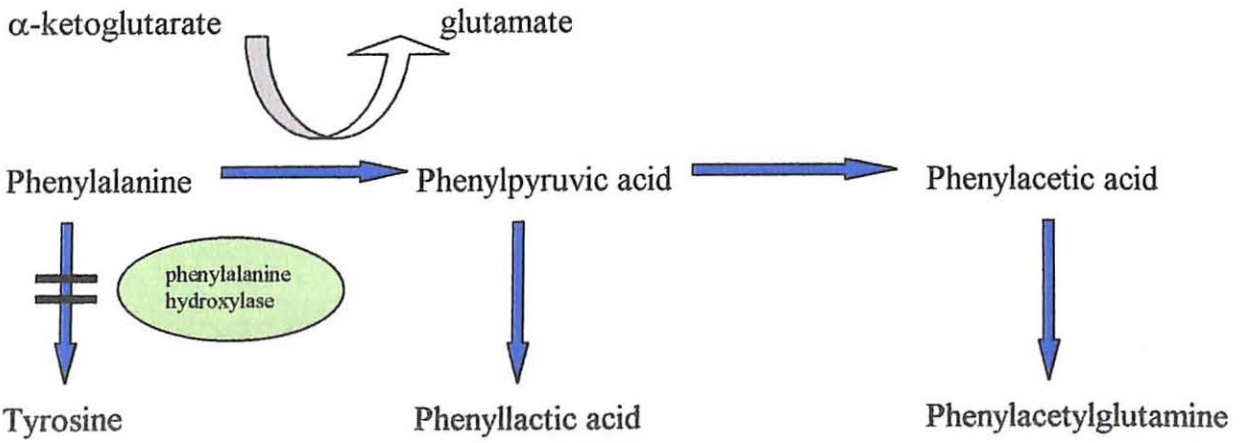

Figure 1.2. Metabolism of phenylalanine. *Phenylalanine hydroxylase* is the site of defect in PKU. The compounds accumulate as a consequence of the defect.

. The pathogenesis of neurotoxicity can be considered from three viewpoints:

1. Deficiency of tyrosine in the brain. Tyrosine is needed for neurotransmitter synthesis in the brain.

2. Effects of phenylalanine on transport and distribution of metabolites in brain. Competition between large neutral amino acids such as tyrosine, tryptophan, leucine, isoleucine, valine and phenylalanine occurs in the brain transport system. Elevated levels of phenylalanine impair the uptake of other amino acids into brain.

3. Effects on neurochemical process. Elevated phenylalanine impairs protein synthesis in the brain. The effect is attributed to polysome disaggregation and inhibition of translation initiation.

1.3.5 DIAGNOSIS OF PKU

In PKU, screening is done initially and the goal is to identify infants with hyperphenylalaninemia. Subsequently, diagnostic tests are performed to identify the phenotype in the particular infant. There are three levels of test in PKU; test at the metabolite level, test at the enzyme level and DNA analysis.

1.3.5.1 Tests at metabolite level

1. Measurement of phenylalanine

   Phenylalanine levels are estimated in whole blood or plasma samples. The normal values are <150μM in neonate and < 120μM in older subjects.

2. Measurement of phenylalanine metabolites

   Phenylpyruvate, a byproduct of phenylalanine can be measured. However, there is a fourfold interindividual variation in the substrate (phenylalanine) concentration in plasma at which the ketoacid is produced in PKU patients.

3. Phenylalanine loading test

   This test is not recommended in newborns. In adults, three days of protein feeding at normal levels is used to classify the phenotype.

4. Plasma phenylalanine response to BH_4

   The BH_4 can be given orally, or through intravenous infusion, which is more reliable. The phenylalanine level must be elevated prior to the test and a fall indicates BH_4 deficiency.

5. Pterin metabolites

   Previously, total pterin activity was assayed based on its cofactor effect on phenylalanine hydroxylation in-vitro. The activity is high in untreated cases of

hyperphenylalaninemia with intact BH_4 synthesis. Now, pterin metabolites can be measured directly by several methods, and one of the most widely used measures total neopterin and biopterin. The amounts and normal values are age-dependent.

6. Neurotransmitter metabolites

   Tyrosine and tryptophan derivatives levels are depressed in BH_4 synthesis disorders.

1.3.5.2 Tests At Enzyme Level

1. *Phenylalanine hydroxylase* (PAH)

   Direct measurement of PAH activity requires liver biopsy. In vivo assay by means of isotopic infusions are now feasible.

2. *Dihydropteridine reductase*

   Activity of this enzyme is measured in many tissues, including liver biopsy material, cultured skin fibroblasts and amniocytes, erythrocytes, leukocytes, platelets and dried blood spots on filter paper.

1.3.5.3 DNA Analysis

DNA samples from venous blood, dried blood spots, buccal cells and cultured skin fibroblasts can be analyzed for mutations.A positive finding does not necessarily indicate a mutation that impairs PAH activity nor does a negative finding exclude a mutation of clinical significance, since no single method detects all possible mutations.

1.3.6 CLINICAL MANAGEMENT

Treatment with low phenylalanine diets should start soon after birth and continued indefinitely. Frequent monitoring is required as phenylalanine is an essential amino acid, and improper therapy can be fatal. In certain countries such as the United States, specific formulae for the diet management and expert nutritional supervision are available through the metabolic programme.

Studies have also been done on the possibility of gene therapy for PKU. Reviews on the current state of gene therapy for PKU showed that of the three basic steps required, two have been accomplished; cDNA clone expressing human *Phenylalanine hydroxylase*, and a phenylalanine hydroxylase-deficient animal model. The third step, establishment of vectors for efficient gene transfer in-vivo is yet to be developed (Eissensmith *et al.* 1996). Recombinant adenoviral vectors, although completely successful in short term, did not persist beyond a few weeks due to an immune response against the adenoviral vector.

1.3.7 SCREENING PROGRAM

In 1953, Bickel reported an effective dietary treatment for reducing brain damage resulting from PKU and suggested that early detection coupled with restricted phenylalanine intake might prevent mental retardation. From this finding, greater emphasis was put on early detection and treatment, which later led to infant screening for PKU. Initially the "wet diaper" test was performed as a preliminary screening technique. The principle of this "wet diaper" test was that any excessive phenylpyruvic acid, a byproduct in the metabolism of phenylalanine excreted in urine, produced a green colour change in the presence of ferric chloride.

Then in early 1960s, mass screening of newborns for PKU was facilitated by Guthrie's development of a simple, effective laboratory procedure. The test was called the bacterial inhibition assay (BIA), and may be performed on specimens collected on filter papers. The Guthrie's method of bacterial testing for phenylalanine and other metabolites created a practical and accurate technique for screening neonates. Blood collected and transported on filter papers is stable, easily transported and causes minimal inconvenience to medical personnel, parents and infants. Once collected, the filter paper of newborn specimen is potentially available for other additional test procedures including DNA analysis (Aldis *et al.* 1993).

The concept of 'early diagnosis' in the field of inborn metabolic errors is not limited to routine screening of every newborn. It also includes selective screening for metabolic

diseases in newborns with severe acidosis, coma, seizure and other symptoms. The concept of early diagnosis has also been expanded to prenatal period, using methods such as amniocentesis, chorion biopsy and fetoscopy with fetal blood sampling. Early clinical diagnosis should remain closely linked to laboratory diagnosis and the practical importance of early diagnosis is in the timely start of treatment (Bickel, 1987).

1.3.7.1 Reasons for screening

Screening for PKU by measurement of phenylalanine level on dried blood spot specimen is recommended for all newborns prior to discharge from nursery. Infants who are tested before 24 hours of age should receive a repeat screening test at two weeks of age by which time the infants would have consumed full protein milk. The benefits of the screening programme are as follows:

a. Benefit to the individual

In PKU, treatment is optimally effective if started soon after birth and before the clinical manifestations of the disease. If not treated during infancy, most infants with PKU develop severe irreversible mental retardation. With routine screening and early treatment, clinical manifestations rarely developed and over 95% of the children develop to normal or near normal intelligence (Feldman, 1993).

b. Benefit to the family

Early diagnosis may benefit the family by reducing stresses caused by a long period of not knowing what is wrong with the infant. The scope of early diagnosis prepares the family for genetic counseling and family planning. Neonatal screening programme also prevents the birth of further affected children.

c. Benefit to society

Newborn screening may have financial advantages in diseases that often result in a chronically sick child with a delayed diagnosis. Any possible saving may be seen as a benefit to society. The expense of screening and treatment is far less than the costs of health care of a handicapped person who may well have a normal life span (Holton, 1988). In Manchester, screening for PKU and treatment of early diagnosed patients were calculated for one year of screening and the result showed that the cost of screening and treatment for these patients were much less than those whom did not receive treatment (Komrower, 1980).

The calculation of the cost and benefits of screening for PKU and treatment of the early diagnosed patients as done by Komrower (1980) is here described. In 1978, eight patients suffering from PKU were detected among 59200 newborn babies screened by paper chromatography, giving the incidence of 1:7400. The total

screening costs were 37 pence per test, screening by paper chromatography being more expensive than by Guthrie's BIA. Calculated on a very comprehensive basis, total costs of diagnosis, treatment, hospital visits and schooling for ten years of the eight patients detected by screening amount to £230000.

The costs of care for six untreated patients with PKU living an average of 45 years are about £760000. These calculations were done on the assumption that two of the eight patients detected by screening will not receive special schooling and residential care. One might die before the age of 5 years and one might not develop severe mental retardation, suffering from mild hyperphenylalaninemia instead of classical phenylketonuria. Considering all these facts the benefit of one year of screening in Manchester area amounts to £53000 which, would raise the benefit considerably.

Table 1.3. Costs of care of six untreated PKU patients in Manchester, England.

Age of the patients (years)	**Place of Rehabilitation**	**Costs(£)**
0-4	Home	-
5-14: 6 x 10 patient-years at £1500 per-annum	Home & special school	90 000
15-45: 6 x 31 patient-years at £3600 per-annum	Residential care	669 600
Total		**759 600**

Note: The calculation was for costs of an average length of life of 45 years.

1.3.7.2 The Efficacy of Screening Test

Blood phenylalanine determination using the Guthrie test has been the principal screening test for PKU for three decades. Although well designed evaluation on sensitivity and specificity of the Guthrie test have never been performed, sensitivity estimates and international experience with its use in millions of newborns suggests that the probability for producing false negative results is rare.

False positive as well as false negative results can occur in PKU screening. The ratio of false positive to true positive in certain situations and population conditions is as high as 32 to 1.8. False positives have been viewed as less important than false negative results because they can be corrected easily whereas false negative results would cause fatal outcome.

1.3.8 OTHER NEWBORN SCREENING PROGRAMMES

Newborn screening programmes have expanded to include many other inherited disorders such as Maple Syrup Urine disease (MSUD), Homocystinuria (HCU), Hypothyroidism and Histedinemia (HE). However PKU remains the most popular treatable metabolic disorder included in screening panels.