COMPARATIVE STUDY BETWEEN THE PHENYLALANINE LEVEL IN UMBILICAL CORD BLOOD AND IN HEEL PRICK BLOOD OF THE NEWBORN

Thesis

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ABSTRACT

Phenylketonuria (PKU) is an autosomal recessive inborn error of phenylalanine (Phe) metabolism resulting from deficiency of phenylalanine hydroxylase (PAH). Most forms of PKU and hyperphenylalaninaemia (HPA) are caused by mutations in the PAH gene on chromosome 12q23.2. Untreated PKU is associated with an abnormal phenotype which includes growth failure, poor skin pigmentation, microcephaly, seizures, global developmental delay and severe intellectual impairment. Neonatal screening for PKU is common throughout the developed world. It represents a model of preventive care in that the screening procedure is simple and intellectual disability is otherwise irreversible. Changes in treatment and care, and in particular the advent of maternal PKU, require regular evaluation of a programme that also impacts on a large healthy population.

Key Words:

1- Phenylketonuria

2- newborn screening

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List of Abbreviations

AAFP	**American Academy of Family Physicians**
AAP	**American Academy of Pediatrics**
BH_4	**Tetrahydrobiopterin**
CS	**caesarian section**
CVD	**Cardiovascular diseases**
HPA	**Hyperphenylalaninaemia**
HPLC ESI-MS	**High performance Liquid Chromatography- electrospray tandem mass spectrometry**
HRSA	**Health Resources and Services Administration**
IEM	**inborn errors of metabolism**
IQ	**Intelligence quotient**
LNAA	**Large neutral amino acids**
mg/dL	**milligrams per decilite**
MHP	**Mild hyperphenylalaninemia**
MRI	**Magnetic resonance imaging**
NBS	**Newborn screening**
NVD	**Normal vaginal delivary**
PAH	**Phenylalanine hydroxylase**
PAH	**phenylalanine hydroxylase**
Phe	**Phenylalanine**
PKU	**Phenaylketonuria**
Trp	**Tryptophan**
Tyr	**Tyrosine**
UAE	**United Arab Emirates**
W A	**westaian Australia**
WHO	**World Health Organization**
µmol/L	**micromols per liter**

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INTRODUCTION

Phenylketonuria (PKU) and its milder variant hyperphenylalaninaemia (HPA) are genetic disorders characterized by a deficiency in PAH an enzyme that is required to metabolize L-Phe to L-Tyrosine (L-Tyr). On the basis of blood Phe concentrations, PAH deficiency can be classified into classic PKU (Phe >1200µmol/L), mild PKU (Phe = 600 - 1200µmol/L) and mild HPA, where blood Phe is elevated above upper reference limit, but <600µmol/L. The decreased PAH activity found in most forms of PKU and HPA are caused by mutations in the *PAH* gene, resulting in a non-functional PAH enzyme ***(Hanley, 2004).***

Untreated PKU is associated with an abnormal phenotype including growth failure, microcephaly, seizures and intellectual impairment caused by the accumulation of toxic by-products of Phe metabolism. The incidence of PKU or HPA is highest amongst Caucasians, occurring in approximately 1 in 10,000 births. PKU can be detected in newborn screening as performed in most Western countries, and early dietary treatment consisting of a low protein diet with Phe restriction can prevent the development of metabolic and pathological sequelae, including intellectual impairment ***(Hanley, 2004).***

Avoidance of brain damage and mental retardation can be prevented if the diagnosis is made early ***(Matalon, 1991).***

PKU is inherited in a simple Mendelian 'autosomal recessive' fashion. PKU disease can take place only if both parents are carriers (PKU carriers have only one defected PKU gene and one sound gene), and if both parents pass the defected PKU gene to their baby. The proportion of two carrier parents having a baby with PKU disease is 1:4 ***(Weatherall, 1991).***

In 1934 Følling discovered PKU disease in Norway however tests for PKU were available only in 1960 after Robert Guthrie developed a PKU screening test called the Guthrie inhibition test. Since then the early diagnosis and proper management of the disease has allowed young people to grow up normally and live full productive lives ***(Guthrie, 1963).***

Routine screening of newborns for PKU is now mandatory in most countries of the world. The Guthrie blood test is normally done before discharging the newborn from hospital. However, it is important to note that this test is not significant until the newborn has taken an ample amount (for 2 or 3days) of the amino acid phenylalanine, which is a constituent of both human and cow's milk ***(Hanley et al, 1997).***

If the PKU test is positive, management and dietary control must start from the first days of life to minimize the possibility of mental retardation and to prevent the accumulation of phenylalanine in them body ***(Wappner et al, 1999).***

Substituting normal milk with phenylalanine free milk and added strained foods low in protein to the infant's diet are the most common dietary control. Blood and urine tests for phenylalanine are considered the most important dietary monitoring and follow-up tool ***(Scriver, 1982).***

Guthrie was the first to demonstrate that blood could be taken from a newborn, absorbed and dried onto standardized filter paper, transported to a testing laboratory and then analyzed for biochemical indicators indicative of congenital disorders, his work on testing for phenylketonuria (PKU) in this way marked the beginning of newborn screening (NBS), in the ensuing four decades, NBS has become a vital public health program preventing debilitating health consequences and providing exceptional health benefits to families and society ***(Therrell, 2006).***

AIM OF THE WORK

The objective of the study is to compare between:-

1. The level of phenylalanine in the umbilical cord blood and in the heel prick blood immediately after delivery and on the third day after delivery
2. The level of phenylalanine in the heel prick blood immediate after delivery and in the heel prick third day after delivery

Chapter (1)

PHENYLKETONURIA

1. History

Phenylketonuria was first described by Asbjorn Følling one of the first Norwegian physicians to apply chemical methods to the study of medicine In 1934, the mother of two intellectually impaired children approached Følling to ascertain whether the strange musty odour of her children's urine might be related to their intellectual impairment **,**The urine samples were tested for a number of substances including ketones. When ketones are present, urine usually develops a red-brown color upon the addition of ferric chloride, but in this instance the urine yielded a dark-green color ***(Følling*** , ***1994)*** .After confirming that the unusual result was not due to any medications and repeating the test every other day for two months, Følling proceeded with a more detailed chemical analysis involving organic extraction and purification of the responsible compound, and determination of its melting point***(*Følling** , ***1994)*** .The basic elements were quantitated by combustion, and an empiric formula of benzene ring ($C_9H_8O_3$) derived ***(*Følling** , ***1994)*** Mild oxidation of the purified substance produced a compound which smelled of benzoic acid, leading Folling to postulate that the compound was phenylpyruvic acid ***(*Følling** , ***1994)*** .There was no change in the melting point upon mixing of the unknown compound

with phenylpyruvic acid thus confirming the mystery compound was indeed phenylpyruvic acid ***(*Følling** , ***1994).***

Følling subsequently requested urine samples from 430 intellectually impaired patients from a number of local institutions and observed a similar result upon addition of ferric chloride, in a further eight individuals ***(Penrose , 1937).*** These eight individuals all presented with a mild complexion (often with eczema), stooping figure with broad shoulders, a spastic gait, and severe intellectual impairment ***(*Følling , *1994)***. Family studies of the affected individuals led to the suggestion of an inherited recessive autosomal trait. Dr Følling published his findings and suggested the name 'imbecillitas phenylpyruvica' relating the intellectual impairment to the excreted substance. Thereafter renamed 'phenylketonuria' ***(Penrose , 1937).***

Our understanding has changed dramatically in the 70 years that have elapsed since the discovery of PKU. Jervis established the metabolic block and enzyme deficiency ***(Jervis 1947),*** and at about the same time, the link between reduced Phe intake and improved prognosis was shown ***(Bickel et al, 1953).*** After the birth of his intellectually impaired son and a niece with PKU, the Canadian Pediatrician Robert Guthrie, changed his research interests and developed screening tests for PKU ***(Guthrie and Susi , 1963)*** In the late 1970s, various groups began investigating the molecular basis of PKU. The most notable recent advance in the study of PKU was the

establishment, in 1996, of the PAH Mutation Analysis Consortium Database ***(Hoang et al, 1996).***

The discovery of PKU by Dr Asbjorn Følling was an important milestone in medicine. The PKU model was used to illustrate how metabolic abnormalities could have neurological effects and how treatment could dramatically affect the clinical manifestations of the disorder. The development of Guthrie's screening test, and dietary treatment, led to the prevention of intellectual impairment in affected children throughout the world. Furthermore, the PKU model has since been used as a template to shed light on over 200 other inborn errors of metabolism ***(Applegarth , 2000).***

Incidenceof PKU

The incidence of PKU in Caucasian populations is between 1 in 10,000 and 1 in 15,000 people. Table(1) shows the variability in incidence in various countries and regions. It has been suggested that the high incidence of PKU in Turkey is due to the high prevalence of consanguinity and the low incidence seen in Finland and Japan is due to a pronounced negative founder effect in Finland and genetic drift in the founding of the Japanese island population ***(Guldberg et al, 1995)***