Republic of Iraq
Ministry of Higher Education
And Scientific Research
University of Kerbala / College of Medicine
Department of Chemistry and Biochemistry

The Utility of Thin Layer Chromatography for Early Detection of Amino Acid Disorder

A Thesis Submitted to the Council of College of Medicine, University of Kerbela, in Partial Fulfilment of the Requirements for the Degree of Master in Clinical Chemistry

By

Muna Ali Hussein

(BSc. In medical and surgery veterinary -University of Kerbala / 2017)

Dr. Rana M Hameed

Ph.D Asst.Prof.in Biochemistry

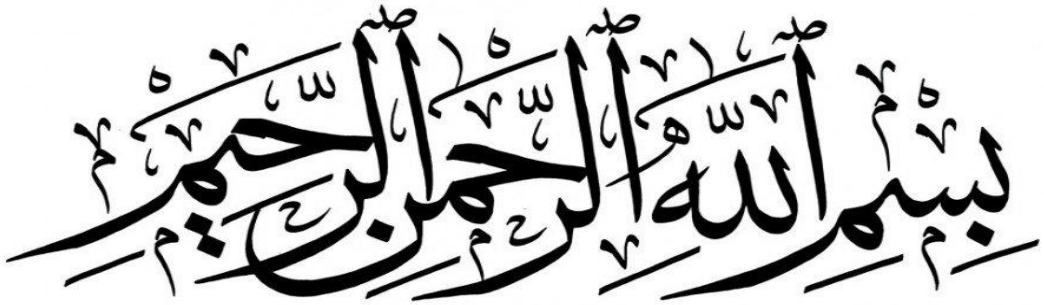
(Supervisor)

Dr. Riadh A Hnewa

Board in Chemical Pathology

(Supervisor)

2021 A.D **1443 A.H**



(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

صدق الله العلي العظيم

سورة المجادله

الآية الحاديه عشر

Supervisor's certification

We certify that this thesis was prepared under our supervision at the College of Medicine /University of Kerbala, as a partial fulfillment for the requirement for the degree of Master in Clinical Chemistry

Dr. Rana M Hameed

Ph.D. Asst.Prof.in Biochemistry

(Supervisor)

Dr. Riadh A Hnewa

Board in Chemical Pathology

(Supervisor)

In the view of the available recommendation, I forward this M.Sc. thesis for debate by the examining committee

Asst Prof. Dr. Rana M. Hameed

Head of Chemistry and Biochemistry Department

College of Medicine / University of Kerbala

Dedication

To my angel in life...to the meaning of love...and to the meaning of tenderness and devotion... (**My beloved mother**).

To whom God has entrusted with dignity and dignity ... To who taught me to give without waiting ... To whom I carry his name with pride ... (**My dear father**)

To those who have succeeded by my side by praying for me at one time and bearing what they could bear with me at another time... (**My brothers …. my life support**).

To all of them I dedicate this humble search to God Almighty asking God to accept it from me and to make it in the balance of my goodness.

Muna

Acknowledgments

In the beginning, I would like to thank God who gave me the strength and patience to complete this work. Also, I would like to thank all the patients who have cooperated with me and also the patients' families. I wish God to write to them healing and wellness.

Then, I would like to express my sincere gratitude to my supervisor, Asst prof. ***Dr. Rana Majeed Hameed*** *for continuous support encouragement, immense knowledge, and Great help in all stages of the search. I would also like to express my great appreciation to* ***Dr. Riadh A Hanewa*** *for his valuable support and advice in this project and provide me with all the facilities to complete this work.*

Also, I would like to thank the Biochemistry department / College of medicine University of Kerbala staff and students, to their kindness and lovely time that I spent with them.

I would like to thank everyone who helped me with even one word, especially ***Dr. Hussein Nehme*** *and my friend* ***(Saba Hameed)****.*

For all so grateful to you

Table of contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Meaning
AAs	Amino acids
AASS	2-aminoadipic acid semi aldehyde synthase
AD	Autosomal Dominant
AKA	α – ketoglutarate
ALT	Alanine amino transferase
AR	Autosomal recessive
AST	Aspartate amino transferase
BCAAs	Branched Chain Amino Acids
BCAT	branched-chain amino acid aminotransferases
BCKDC	branched-chain α-keto acid dehydrogenase enzyme complex
BH4	tetrahydrobiopterin
BMI	Body mass index
CACT	Carnitine acyl-carnitine transferase
CBS	cystathionine-β-synthase
CDO1	Cysteine dioxygenase 1
CPT II	Carnitine palmitoyl transferase II
CPTI	Carnitine palmitoyl transferase I
CSF	Cerebral spinal fluid
DNPH	Dinitrophenyl hydrazine
EAAs	Essential amino acids
FAODs	Fatty Acid Oxidation Disorders
GA I	Glutaric aciduria type I
GABA	γ-aminobutyric acid
GCS	glycine cleavage system

GSD	Glycogen storage diseases
GSH	Glutathione
HPLC	high-performance liquid chromatography
IEM	Inborn errors of metabolism
INR	International Normalized Ratio
MCAD	Medium chain acyl coA dehydrogenase
MRC	mitochondrial respiratory chain
MSUD	Maple Syrup Urine Disease
NAD	Nicotinamide adenine dinucleotide
NBS	Newbern Screening
NKH	Nonketotic Hyperglycinemia
OADs	Organic acidurias disorders
OXPHOS	oxidative phosphorylation
PAH	phenylalanine hydroxylase
PBDs	Peroxisome biogenesis disorders
PGD	Preimplantation genetic diagnosis
PKU	Phenylketonuria
PLP	Pyridoxal phosphate
Rf	Retention factor
SAM	S-adenosyl methionine
SCADD	Short-chain acyl-CoA dehydrogenase deficiency
SHMT	Serine hydroxyl methyl transferase
TLC	thin layer chromatography
TMS	Tandem mass spectrometry
UCDs	Urea cycle disorders
VLCAD	Very long chain acyl coA dehydrogenase
VLCFs	Very long-chain fatty acids
WHO	World Health Organization

Summary:

Metabolism is organized into pathways where the components are transformed by the action of different enzymes that catalyze the transformation of the substrates (synthesis or degradation). When one or more of these enzymes are not working properly or are absent, metabolic disorders can occur. Most inborn error of metabolism are inherited in an autosomal recessive manner.

The overall incidence of inborn errors of metabolism is estimated to range from 1:800 to 1:1000 live births, although the incidence is probably much higher given difficulties in clinical diagnosis and limitations in diagnostic testing. The incidence and prevalence of IEM were vary in different countries. Classification is based on the affected metabolic process or enzymes involved in generating toxic substances such as the individual disorders of amino acid, Disorders of carbohydrate metabolism, Disorder of lipid metabolism and Disorders in the metabolism of purines and pyrimidines.

The aim was introduce a separation a TLC method of standard amino acids that could be effective for the detection of amino acid disorders by producing distinguishable strategies based on silica gel TLC plates.The present work included a cross section study. Samples were selected, from patients attending the rare diseases unit, Kerbala teaching hospital for children. and the standardization of 20 amino acid was done by TLC (10 time for each amino acid). After that, separation of amino acid in the serum and urine were performed for patient group.The study were included 56 samples, ages ranging from 7 day to 15 years. The patient samples were divided as follows :Neonate of age < 1month were 7 ,new-born aged from 2-12 months numbered 39, children aged >1year were 10, females were 22 and males represent 34, our study shows that 41% of the suspected IEM patients were with sign of jaundice, and 32% ,29% ,18%,16% ,5%, 5% with flatulence, vomiting ,

diarrhea , acidosis, dehydration and hepatomegaly respectively. Results was shown about 50% , 41%, 39% ,36% ,18% of suspected IEM patients were with hypotonia ,spasticity, intellectual delay, epileptic seizures and lethargy respectively .

Standardization of amino acids on TLC was performed based on different strategies: the study results were established different strategies showing good demonstration of the standard AA throughout the retention factor (Rf) (mean and /or range), the arithmetic retention factor resulted in subdivided of AA into 6 group accordingly. Also the sstandardization of AA were separated based on producing various distinguishable colors/ shapes. The above three methods help to identify amino acids in the serum and urine samples. It has been reported that one third of the IEM were amino acid disorders, and these disorders must be diagnosed and treated as early as possible to prevent the unpleasant complication. Generally, to get diagnosis, HPLC & TMS must done for the suspected patients if have amino acid disorder, both methods are expensive, time consuming and available only in a few locations in Iraq. In order to exceed these adverse effects, this study suggested starting with TLC (easy , unexpensive , time effective) which could help to screen suspected patients with amino acid disorders, then the more specific and sensitive methods (HPLC & TMS) could be used only to prove diagnosis.

Chapter One

Introduction

1. Introduction and Review of Literature

1.1. Inborn errors of metabolism:

The term metabolism encompasses the net result of a multitude of complex biochemical processes that occur in living organisms to maintain cellular activities vital to sustain life. These processes are organized into specific metabolic pathways with the primary function of maintaining daily life activities. Each pathway depends on certain substrates and specific enzymes to ensure smooth functioning (1). Normal metabolism should result in normal growth and development, good energy production, elimination of waste molecules and correct nutrient distribution in the body. Metabolism is organized into pathways where the components are transformed by the action of different enzymes that catalyze the transformation of the substrates (synthesis or degradation). When one or more of these enzymes are not working properly or are absent, metabolic disorders can occur (2). Most metabolic disorders are inherited as autosomal recessive traits. Autosomal recessive inheritance and enzyme deficiency are features typical for an inborn error of metabolism. Inborn errors of metabolism (IEM) are disorders genetically determined by deficiencies of an enzyme involved in the synthesis, transport or degradation of molecules in a metabolic pathway. The occurrence of a block in one stage of a pathway results in the lack or excess of a particular substance and may, in addition, interfere in an alternative metabolic pathway (3).

1.2. General Biochemical defects and pathophysiology:

Most IEMs are inherited in an autosomal recessive (AR) manner (4). In AR diseases, phenotypes will only manifest if both parental copies of the mutated allele are inherited. Homozygote gene mutation inheritance can occur by either consanguineous marriage or by a random mutation in the second

allele in heterozygote parents. Some IEMs are inherited as X-linked alleles, and therefore have higher incidence rates in males. Due to variable random X-chromosome inactivation syndrome in females, X-linked IEMs diseases can have highly variable manifestations from one tissue to another and from one female (if so affected) to another. A minority of IEMs are inherited in an autosomal dominant (AD) pattern. Another rare mode of inheritance is IEMs linked to mitochondrial DNA only of maternal origin, as in subsets of respiratory chain disorders (Figure 1.1) (5).

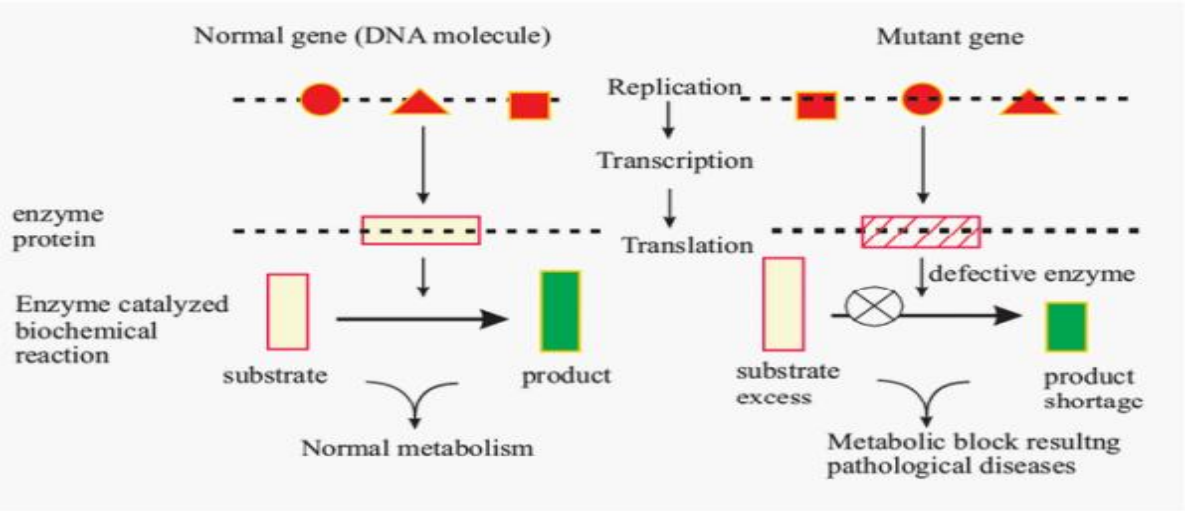


Figure 1-1: Inborn errors of metabolism (6).

1.3. History of inborn errors of metabolism:

The term 'inborn errors of metabolism' (IEM), which is also referred to as congenital metabolic diseases or inherited metabolic diseases was first coined by a British physician Archibald Garrod (1857–1936) to describe the hereditary deficiency or alteration in enzyme reactions in the early 20th century (7). He described alkaptonuria as a rare, hereditary, and recessive metabolic defect in tyrosine degradation that results in the accumulation of homogentisic acid. Garrod also extended his studies into albinism, cystinuria, and porphyria However, medical society at that time failed to recognize the significance of Garrod's observations due to confusion regarding the

expansion and application of approaches to IEM. The resulting period of inactivity was mainly due to a lack of technologies capable of enabling investigations at the cellular and molecular level, not until 39 years after Garrod first described IEM did Beadle and Tatum establish the foundation of biochemical genetics, demonstrating that each biochemical process is controlled by a specific gene or protein (8).

1.4. Epidemiology of IEM:

The overall incidence of inborn errors of metabolism is estimated to range from 1:800 to 1:1000 live births, although the incidence is probably much higher given difficulties in clinical diagnosis and limitations in diagnostic testing (9). The incidence and prevalence of IEM were vary in different countries. The frequency of IEM is increased in the countries or regions where the rate of consanguineous marriages is high. Most of the people there were belonged to tribes with a high frequency of consanguineous marriages. However, there was limited data about this rate in Karbala. In general, a high rate of consanguinity was recorded in Iraq reaching an average of 47–60%, which may be related to the social, cultural, religious, and political factors. To date, in Iraq only two provinces (Baghdad and Karbala) had applied a new born screening (which was started at 2013) no clear data presented from Iraq**.** Up to now, more than 500 IEMs have been detected. Although individual IEM is rare, the incidence of overall IEMs is high and varies dramatically in different countries and regions**,** For example locally, the incidence of IEMs was reported to be by the number of participants who were screened in Misan town over one year (from 1 April 2017 to 1 April 2018) was 112, it has been recorded that 20 cases which about 18 % were identified and confirmed to have IEM (10). Other study in Baghdad from December 2009 to December 2012 which included 1758 participants were selected from Al- Emamain AL Kadhemyian and the children's welfare teaching hospitals, they reported

positive cases of IEM which were around 224 (12.7 %) cases (11). While in Mosul and Kurdistan region (from January 2018 to January 2020) a study conducts on 3000 participants and only (4.4 %) of cases were reported and verified to have IEM. (12). In the Arab countries the overall frequency of metabolic disorders were: In Saudi Arabia, Al Ahsa (Apr 2006 – Apr 2009) was 48 case /38 001 (13). Oman, AlKhoudh (May1998–Jul2008) was 119 case /1100 (14). Bahrain (Jan 2008 – Dec 2011) 25 case /66 565 (15). Egypt (Jun 2008 – Jun 2013) 203case/3380 (16).

1.5. Classification of IEM:

The classification of IEM is challenging. Several classifications currently exist. The favourable classification is to small molecules diseases such as carbohydrate, protein, lipid and nucleic acids and large molecules diseases such as lysosomes, mitochondria, peroxisomes and cytoplasm (17). Another classification is based on the affected metabolic process or enzymes involved in generating toxic substances such as the individual disorders of amino acid (e.g., maple syrup urine disease, organic acidemias, and urea cycle defects) Disorders of carbohydrate metabolism (e.g., galactosemia and glycogen storage disorders) ,Disorder of lipid metabolism (e.g., fatty acid oxidation defects [medium chain acyl-CoA dehydrogenase deficiency]) ,Disorders in the metabolism of purines and pyrimidines (18). Often Metabolic disorders also can be subdivided into three groups based on their clinical and physiologic characteristics .**the first group of disorders** lead to excessive accumulation of a substance or compounds with intoxicating effects (eg, vomiting or obtundation), as seen in aminoacidopathies, organic acid disorders and urea cycle defects ,appearance at Birth usually normal .**The second group of disorders** directly affect energy metabolism causing symptoms in more metabolically active organs such as the brain (eg, coma, brain malformations), as seen in mitochondrial disorders, fatty acid oxidation disorders, appearance

at birth sometimes normal but may affect metabolically active tissues (brain malformation).**The third group of disorders** involves defects in the catabolism of complex molecules in cell organelles, as seen in lysosomal storage disorders or peroxisomal disorders. Appearance at birth sometimes normal, but some present with dysmorphology or organomegaly (19).

1.6. General Clinical Overview of IEM:

1.6.1. Organic Acidemias or Organic acidurias disorders (OADs):

A group of inherited disorders of intermediary metabolism resulting from deficiency of an enzyme or a transport protein required in one of the several cellular metabolic pathways involved in the catabolism of amino acids, carbohydrates or lipids. These deficiencies result in abnormal accumulation of organic acids in the body and their abnormal excretion in urine (Figure 1.2) (20).

1.6.2. Carbohydrate Metabolism Disorders:

Carbohydrate disorders may include deficiencies of enzymes involved in the metabolism of glycogen, galactose, and fructose. These diseases can be broadly sub classified as diseases causing liver dysfunction, diseases affecting muscle and liver, and diseases affecting only muscle. Galactosemia, glycogen storage diseases and hereditary fructose intolerance are common examples of carbohydrate metabolism disorders (21).

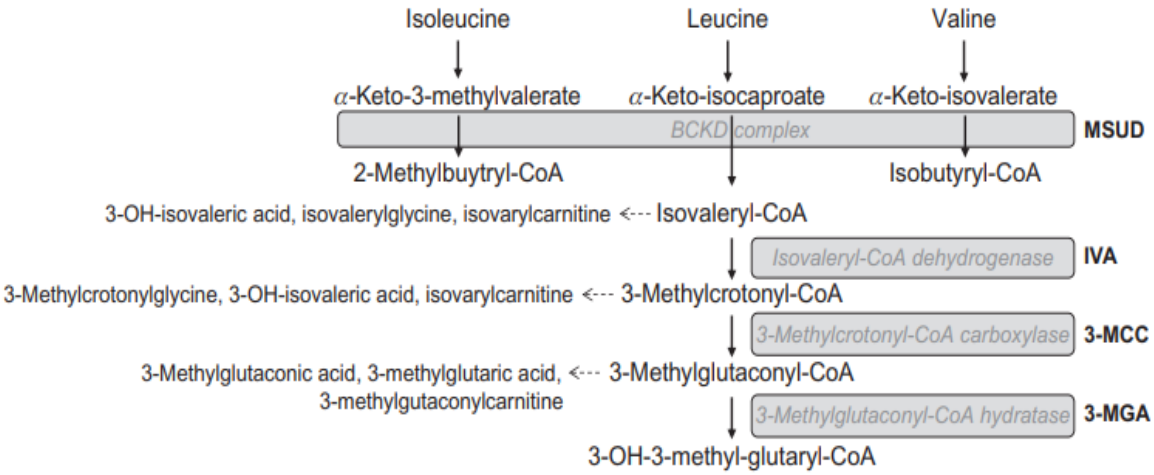


Figure 1-2: Disorders of branched-chain amino acid metabolism. Bold: inborn errors of metabolism resulting from deficiencies in enzymes. Dashed arrows: abnormal production of metabolites. 3-MCC, 3-Methylcrotonyl-coA carboxylase; IVA, isovaleric aciduria; MGA: 3- methylglutaconic aciduria; MSUD, maple syrup urine disease.

1.6.3. Urea Cycle Disorders (UCDs):

The urea cycle is a metabolic pathway for the disposal of excess nitrogen, which arises primarily as ammonia .UCDs comprise diseases presenting with hyperammonemia that arise in either the neonatal period (about 50% of cases) or later. Congenital defects of the enzymes or transporters of the urea cycle cause the disease (figure 1.3) (21).

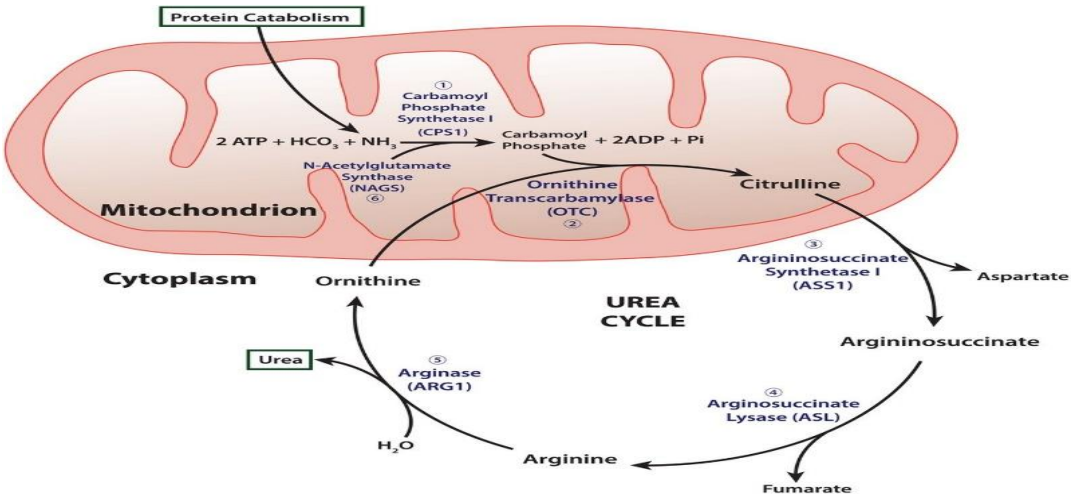


Figure1- 1: The urea cycle produces urea from the nitrogenous waste products of protein metabolism. The six enzymes of the pathway are numbered 1–6, with their associated gene in brackets (22).

1.6.4. Fatty Acid Oxidation Disorders (FAODs):

Fatty acid oxidation produces acetyl-CoA, which supplies energy to other tissues when glycogen stores are depleted. The medium- and short-fatty acids are transported directly into the cytosol and mitochondria. The long-chain fatty acids are conjugated to carnitine and transported across the mitochondrial membrane and released as acyl-CoA to be used in the β-oxidation pathways .Mitochondrial fatty acid oxidation disorders comprise 4 groups: (1) disorders of the entry of long-chain fatty acids into mitochondria, (2) intramitochondrial β-oxidation defects of long-chain fatty acids affecting membrane bound enzymes, (3) β-oxidation defects of short- and medium-chain fatty acids affecting enzymes of the mitochondrial matrix , (4) disorders of impaired electron transfer to the respiratory chain from mitochondrial β-oxidation (Figure 1.4) (23).

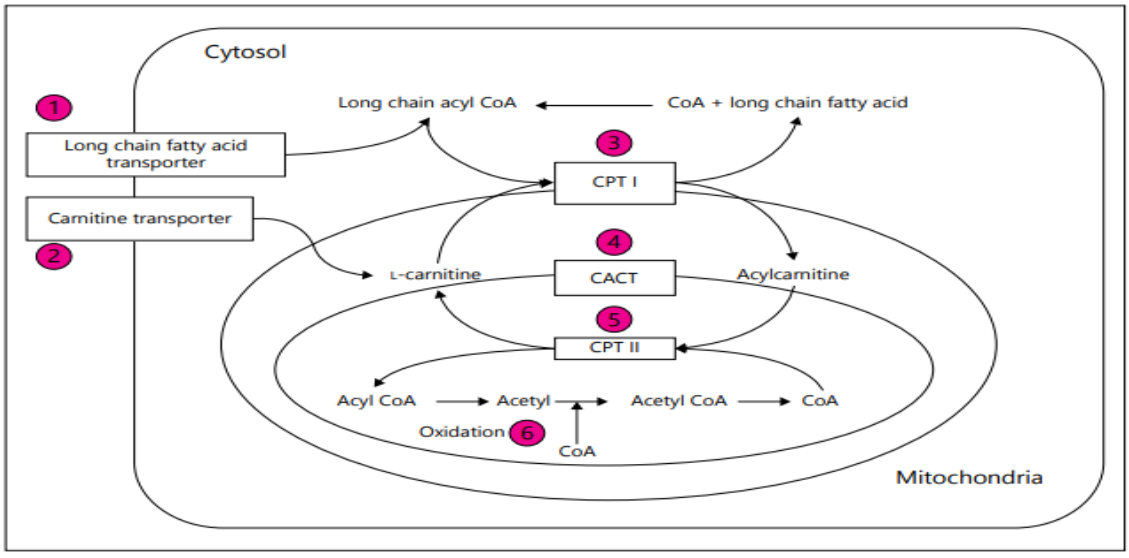


Figure1-2: Long chain fatty acid oxidation. The different sites of deficiencies causing FAODs are listed: (1) long-chain fatty acid transport or binding effect, (2) carnitine uptake defect, (3) CPT I deficiency, (4) CACT deficiency, (5) CPTII deficiency, (6) VLCAD, MCAD and SCAD deficiencies (24).

1.6.5. Mitochondrial Disorders:

Are characterized by a high genetic, biochemical, and clinical complexity that arise from the dysfunction of the oxidative phosphorylation (OXPHOS), the essential, final pathway for aerobic metabolism. Such impairment is caused

by mutations in genes encoding for proteins involved in mitochondrial respiratory chain (MRC) biogenesis (25).

1.6.6. Lysosomal Storage Disorders:

Lysosomes contain more than 50 acid hydrolases that can degrade unwanted complex molecules such as mucopolysaccharides, sphingolipids, glycoproteins, etc., into molecules which could be used by the body again. Therefore, lysosomes can be regarded as the recycling centres of the body. Lysosomal storage diseases are caused by the inability of the lysosome to degrade one or more of the complex substrates in its lumen or membrane due to defects in lysosomal enzymes, enzyme receptors, membrane proteins, activator proteins, or transporters, so that characterized by a progressive accumulation of undigested substrates and cell death (26).

1.6.7. Peroxisomal Disorders:

The peroxisome is a cellular organelle with disparate functions, including oxidation of very long-chain fatty acids (VLCFAs). Defects in peroxisomal function or assembly can result in several severe neurodegenerative disorders. Adrenoleukodystrophy that results from a deficiency in peroxisomal oxidation of VLCFAs (27).

1.6.8. Purine and Pyrimidine Metabolism Disorders:

Purine and pyrimidine bases, nucleosides and nucleotides are essential components of the nucleic acids DNA and RNA, and are associated with metabolic regulation, synthesis of numerous biomolecules and other vital processes in cell physiology (28). Genetic defects of purine and pyrimidine metabolism represent a group of relatively new disorders (29)**.**

1.6.9. Porphyria disorder:

The porphyrias are a group of rare metabolic disorders—either inherited or acquired along the hem biosynthetic pathway. Each type of porphyria is a

result of a specific deficiency in one of the enzymes involved in the pathway and, accordingly, is characterized by a specific pattern of accumulation of hem precursors and typical clinical manifestations (30).

1.7. Amino acids (AAs):

The amino acids that are incorporated into mammalian protein are α-amino acids. This means that amino acids have a carboxyl group, an amino nitrogen group, and a side chain attached to a central α-carbon (Figure1.5) Proline is the only exception, being unique among the 20 protein-forming amino acids in that the amine nitrogen is bound to two alkyl groups, thereby making it aα-imino acid. Functional differences among the amino acids lie in the structure of their side chains (31). (AAs) are the building blocks of proteins, including structural proteins and enzymes. They also form the backbones of critical nitrogen-based compounds such as cytochromes, heme, hormones, melanin, neurotransmitters, nucleotides/nucleic acids and others. (AAs) are used efficiently by the body by multiple mechanisms such as recycling, transamination, or energy production, When needed, the carbon skeletons can be used to produce glucose or its derivatives (such as glycogen or fatty acids) to support plasma glucose levels or provide energy during fasting. Dietary proteins are key sources of essential amino acids in mammals and humans, while non-essential amino acids can by synthesized endogenously. Nine amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) are considered as "essential" essential in the diet as humans are unable to synthesize them endogenously. Most of the non-essential amino acids such as alanine, arginine, asparagine, aspartate, glutamate, glutamine, glycine, proline and serine, are synthesized from glucose; while tyrosine is synthesized from the metabolism of phenylalanine, and cysteine from the metabolism of methionine (32).

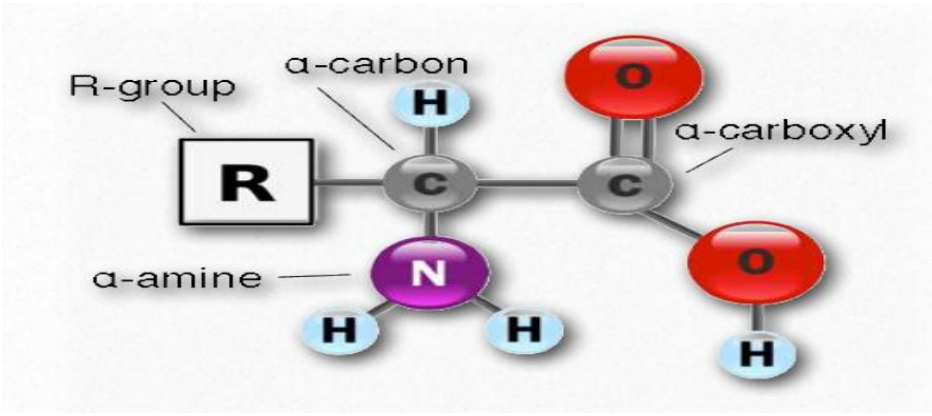


Figure 1-3 : An amino acid: an amino (NH2) group, R a carboxyl group (COOH) and a side chain attached to a central α-carbon (R) (33).

1.7.1. Simple, Hydroxyl and Sulfur-containing Amino Acid

Glycine (Gly): Is the most important and simple amino acid with no L or D chemical configuration, nonessential and glucogenic amino acid in humans. Generally, glycine is syntheses from choline, serine and threonine through interorgan metabolism in which kidneys and liver are the primarily involved .Glycine acts as precursor for several key metabolites such as creatine, glutathione, heme, purines, and porphyrins. Degradation of glycine in humans is done via three pathways: (1) D-amino acid oxidase converting glycine into glyoxylate, (2) serine hydroxymethyltransferase (SHMT) converting glycine into serine, and (3) deamination and decarboxylation by glycine cleavage enzyme system (GCS). The physiological importance of the GCS in degradation of glycine is characterized by its defect in humans, which results in glycine encephalopathy. After phenylketonuria, glycine encephalopathy is the most frequently occurred inborn error of amino acid metabolism (34).

Serine (Ser): Is an aliphatic hydroxyl and polar amino acid, which is non-essential and glucogenic (35). L-serine is biosynthesized in the mammalian central nervous system from 3-phosphoglycerate and serves as a precursor for the synthesis of the amino acids glycine and cysteine. Mutations in the metabolic enzymes that synthesize L-serine have been implicated in various human diseases (36).

Alanine (Ala): Is a non-essential, glucogenic amino acid and Non-polar, aliphatic residues (37). Alanine can be formed by transamination of pyruvate. The enzyme is alanine amino transferase (ALT).This reaction requires pyridoxal phosphate (PLP). ALT level in blood is increased in liver diseases. Under conditions of starvation, the glucose alanine cycle has special metabolic significance. Alanine is quantitatively the most important amino acid taken up by the liver from peripheral tissues, particularly from skeletal muscle. It forms a major participant in inter-organ transport of nitrogen (38).

Threonine (Thr): Is an essential and polar uncharged R group's amino acid. It is glucogenic. Threonine does not directly undergo transamination, but undergoes deamination forming alpha ketobutyric acid by the enzyme threonine dehydratase. In turn, alpha ketobutyric acid can be oxidative decarboxylated to propionyl CoA, and then converted to succinyl-CoA, which enters the gluconeogenesis pathway. The -OH group of threonine residue in protein serves to provide a site for phosphorylation. This -OH group also serves for combining carbohydrate residues to proteins, to make glycoproteins (39).

Methionine (Met): Is sulfur-containing, nonpolar R groups, essential, glucogenic amino acid. Degradation of methionine results in the synthesis of cysteine. Methionine is converted to S-adenosylmethionine (SAM) by methionine adenosyltransferase. SAM is the methyl donor for all the transmethylation reactions (Creatine, Epinephrine, Choline and Melatonin) improper transformation of methionine can lead to atherosclerosis due to the accumulation of homocysteine (40).

Cysteine (Cys): Is one of the non-essential, glucogenic amino acid. Formation of cysteine is by using the carbon skeleton contributed by serine and sulfur originating from methionine, and Cysteine mainly exists as cysteine (41). The

sulfur-containing amino acid cysteine supports various important cellular pathways including protein synthesis, glutathione (GSH) synthesis, taurine synthesis, and sulfate production .GSH plays important roles in cellular antioxidant defense and detoxification, Cysteine dioxygenase type-1 (CDO1) catalyzes the irreversible conversion of cysteine to cysteine sulfinic acid, which is the major cellular cysteine elimination mechanism, abnormally elevated cysteine can cause oxidative stress and cytotoxicity in certain cell type (42).

1.7.2. Acidic and Basic Amino Acids Metabolism

Glutamic acid (Glu): Is a glucogenic, negative charge, acidic amino acid due to its two carboxyl groups (α- and γ-carboxyl) and a neurotransmitter in human body, plays a central role in the metabolism of amino acids which is generated during transamination reactions. Glu is a common precursor of many organic compounds. These organic compounds include protein amino acids (glutamine: Gln; proline: Pro; arginine: Arg; and histidine: His), non-protein amino acid (γ-aminobutyric acid, GABA), antioxidant tripeptide (glutathione, GSH), heme, chlorophyll (43).

Glutamine (Gln): Is a nonessential, glucogenic amino acid. It could be synthesized from glutamic acid, the amidation of glutamic acid to glutamine catalyzed by glutamine synthetase. Glutamic acid can react with a molecule of NH_3 in presence of ATP. This reaction is important in ammonia trapping in brain as well as for transport of ammonia in a nontoxic form. Major fate of glutamine is to be hydrolyzed to glutamate and NH_3. Glutamic acid is then deaminated to α-ketoglutarate and enters TCA cycle for further catabolism (44).

Aspartic acid (Asp): Is a nonessential, glucogenic amino acid. Aspartate, on transamination gives rise to oxaloacetate, which initiates the TCA cycle.

Aspartate amino transferase (AST) transfers the amino group of aspartate to α-ketoglutarate (AKG) to form oxaloacetate .The AST activity levels is increased in cardiac ischemia and in hepatic diseases. Participates in a range of important cellular functions such as the urea cycle and the malate-aspartate shuttle, which transports nicotinamide adenine dinucleotide (NAD) reducing equivalents between the cytoplasm and mitochondrial matrix (45).

Asparagine (Asn): : Is a non-essential, glucogenic amino acid, Aspartate reacts with ammonia to form asparagine; Asparagine can be hydrolyzed to aspartate and NH_3 by asparaginase (46). The defect is attributed to the defect in renal tubular reabsorption of glutamate and aspartate can be cause Dicarboxylic Aminoaciduria (47).

Lysine (Lys): Is an essential basic amino acid. It does not undergo transamination. Lysine is predominantly ketogenic. The degradation of L-lysine may occur via the mitochondrial saccharopine pathway, the first two steps are catalyzed by a bifunctional enzyme, the mitochondrial 2-aminoadipic acid semi aldehyde synthase (AASS) and (AASS) deficiency leads to hyperlysinemia (48). It is involved in synthesis of collagen, carnitine and elastin (49).

Arginine (Arg): Semi-essential amino acid, contains guanidinium group. It is a highly basic. Arginine is glucogenic because it can be metabolized into AKG and enter the citric acid cycle. It plays a critical role in cytoplasmic and nuclear protein syntheses, the biosynthesis of other amino acids, ornithine and creatine synthesis, and the urea cycle. In one of its most important roles, L-arginine serves as a precursor for the biosynthesis of nitric oxide (NO) (50).

1.7.3. Aromatic Amino Acids Metabolism

Phenylalanine (Phe): Is an essential, aromatic amino acid; it is partly glucogenic, partly ketogenic and it converted to tyrosine by phenylalanine hydroxylase. A defect in this enzyme leads to phenyl-ketonuria (PKU) (51).

Tyrosine (Tyr):` Is an aromatic non-essential amino acid, synthesized from phenylalanine, Tyrosine is partly glucogenic and partly ketogenic. Tyrosine is a precursor of acetyl-CoA and fumarate, catecholamine hormones, the neurotransmitter dopamine, and the pigment melanin and thyroxine (52).Tyrosinemia is associated with a metabolic defect in the most enzymes in the tyrosine catabolic pathway (53).

Tryptophan (Try): Is an aromatic, essential amino acid with an indole ring and is partly glucogenic and partly ketogenic, the major metabolic fate of tryptophan is to be oxidized by tryptophan pyrrolase . It is the precursor of serotonin and Melatonin, Tryptophan is degraded by the kynurenine pathway to NAD and other products with several of the intermediates being toxic such as quinolinic acid. Nutritional pellagra therefore occurs only if diets are deficient in both niacin and Trp (54).

Histidine (His): It is an essential basic glucogenic amino acid, has an imidazole ring. Histidine is first non-oxidatively deaminated by histidase to form urocanic acid. The deficiency of histidase leads to accumulation of histidine in blood and body fluids and increased excretion of imidazole pyruvic acid in urine and it is the precursor of Histamine (55).

Proline (Pro): Is a non-essential, glucogenic amino acid. Proline can be endogenously synthesized from either glutamate or ornithine, Alterations in proline metabolism have been described in various human diseases, such as hyperprolinemia type I (56).

1.7.4. Branched Chain Amino Acids (BCAAs):

Valine (Val): is glucogenic; Leucine (Leu) is ketogenic while Isoleucine (Ile) (I) is both ketogenic and glucogenic. All the three are essential amino acids (EAAs). Leucine is the major ketogenic amino acid. Unlike most other EAAs, BCAAs catabolism initially catalyzed in extrahepatic tissues by the branched-chain amino acid aminotransferases (BCAT) and branched-chain α-keto acid dehydrogenase enzyme complex (BCKDC).The BCAAs metabolites are further converts to final products (acetyl-CoA and Succinyl-CoA) via a series of enzymatic reactions and participate in metabolism of tricarboxylic acid cycle and BCAAs is a major player of the branched-chain fatty acid synthesizing system. (57). Dysregulation of BCAAs metabolism is associated with a range of diseases such as Maple Syrup Urine Disease (MSUD) caused by BCKDC dysfunction (58). One class of IEMs that is caused by the abnormal changes of physiological amino acids is called amino acidopathies. Common aminoacidopathies are metabolic disorders, which are caused by defective metabolic pathways due to the deficiencies of functional enzymes (34).

1.8. Amino Acids Disorders in IEM

1.8.1. Phenylketonuria (PKU):

It's usually caused by a deficiency of the hepatic enzyme, phenylalanine hydroxylase (PAH). The enzyme catalyzes the hydroxylation of phenylalanine to tyrosine, the rate limiting step in phenylalanine catabolism in the presence of the cofactor tetrahydrobiopterin (BH4) and several enzymes (dihydropteridine reductase and pterin 4 alpha -carbinolamine) that serve to regenerate BH_4 (Figur1.6). Enzyme deficiency leads to accumulation of phenylalanine resulting in hyperphenylalaninaemia, increased phenyl ketones

(hence phenylketonuria) (Figure1.7) and a decrease in myelin formation, dopamine, norepinephrine, and serotonin production (59).

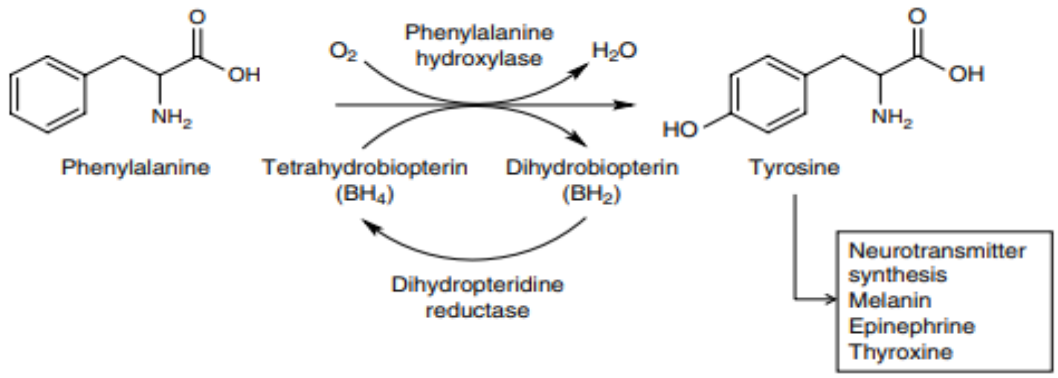


Figure 1-4: Hydroxylation of phenylalanine (60).

1.8.2. Hereditary Tyrosinaemias

Impaired degradation of the amino acid tyrosine is a feature of several acquired and genetic disorders .Tyrosine is catalyzed by a series of five enzymatic reactions yielding acetoacetate (ketogenic) and fumarate (gluconeogenic). Only liver and renal proximal tubules express the complete pathway and contain all the enzymes required for tyrosine catabolism. Inborn errors of tyrosine degradation include (1) Tyrosine aminotransferase deficiency (Tyrosinemia type II), (2) 4-hydroxyphenylpyruvate dioxygenase deficiency (Tyrosinemia type III and Hawkinsinuria), (3) Homogentisic acid oxidase deficiency (Alkaptonuria), (4) Fumarylacetoacetase (FAH) deficiency (Tyrosinemia type I) (Figure1.7) (61).

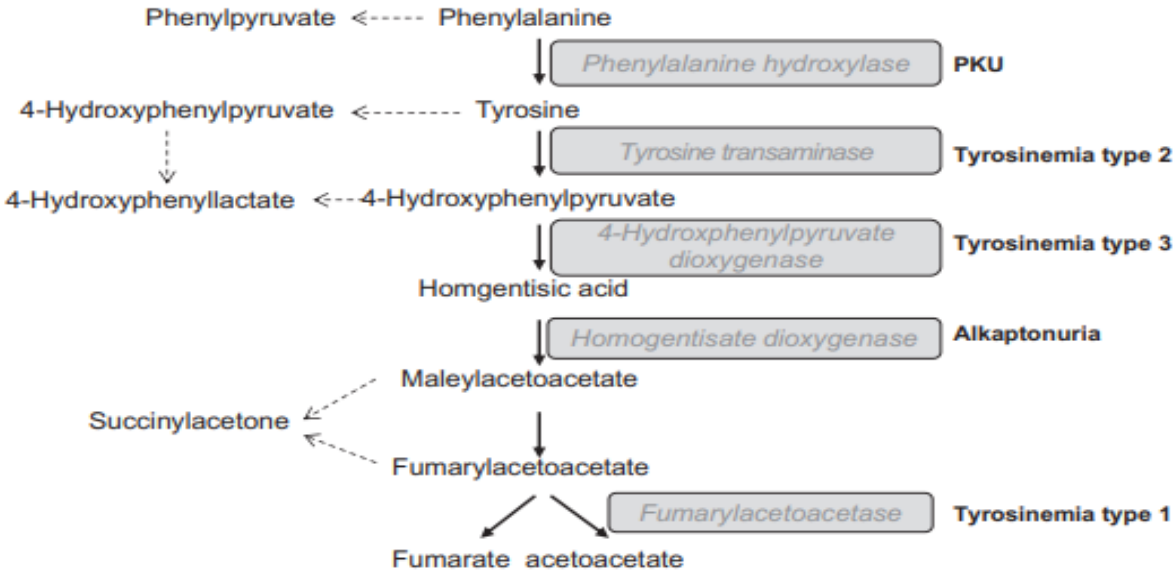


Figure 1-5 Disorders of phenylalanine and tyrosine metabolism. Bold: inborn errors of metabolism resulting from deficiencies in enzymes. Dashed arrows: abnormal production of metabolites. PKU, Phenylketonuria (63).

1.8.3. Alkaptonuria:

The metabolic disease caused by deficiency of homogentisic acid oxidase, due to this defect, homogentisic acid, which is produced during the metabolism of tyrosine cannot be converted to maleylacetoacetate (Figure1.7). Accumulates in tissues, and is excreted in the urine. This enzyme primarily can be found in the liver and kidney. It requires oxygen, ferrous iron, and sulfhydryl groups to open the ring of homogentisic acid. Accumulation of homogentisic acid and its metabolites causes ochronosis with darkening of cartilaginous tissues and bone, arthritis and joint destruction (62).

1.8.4. Maple syrup urine disease (MSUD):

Is a disorder of branch chain amino acids metabolism caused by a deficiency of branched-chain α-keto acid dehydrogenase complex .MSUD characterized biochemically by elevated plasma branched-chain amino acids (leucine, isoleucine, valine) and α-ketoacids in urine (64).

1.8.5. Homocysteinuria:

Is a disorder of either a nutritional deficiency or an inherited defect in the enzymes involved in the remethylation and transsulfuration pathways of methionine metabolism. Classical homocystinuria is due to the deficiency of cystathionine-β-synthase (CBS) enzyme which is the most common cause of homocysteinuria. CBS is a pyridoxal-5′-phosphate-dependent enzyme which catalyzes the conversion of homocysteine and serine to cystathionine in the transsulfuration pathway of homocysteine metabolism to form cysteine. The characteristic amino acid profile of CBS deficiency shows high levels of homocysteine and methionine, and low levels of cystathionine and cysteine in the blood and urine (65).

1.8.6. Nonketotic Hyperglycinaemia (NKH):

Is a disorder, also known as glycine encephalopathy, results from the accumulation of glycine in all body tissues, due to inactivity of the glycine cleavage system (GCS). It is the second most common disorder of amino acid metabolism**.** The GCS converts two-carbon glycine into ammonia and carbon dioxide, and transfers one-carbon unit to folate as methylenetetrahydrofolate. Without a fully functional GCS, glycine accumulates in tissue, plasma, urine, and CSF (66)**.**

1.9. Clinical Approach to IEM:

Inborn errors can present with a wide spectrum of clinical signs and symptoms at any age. Mainly the signs and symptoms resulting from IEM can be divided into the early-onset and late-onset forms (67).

1.9.1. Early-Onset Signs and Symptoms of IEM :

Disorders such as non-ketotic hyperglycinaemia, glutaric aciduria type II, and some of the mitochondrial diseases, and urea cycle defects (UCDs) are known to begin during the prenatal period (Table1.1) (68). During the new-born period, coma, hypotonia or hypertonia, seizures, organomegaly are the

most commonly seen clinical symptoms. After a certain healthy period following birth, the signs and symptoms typically begin and might characteristic of "intoxication type" IEM. The severe forms of aminoacidopathies, branched-chain organic acidurias, UCDs, mitochondrial disorders, peroxisomal defects, and ketolysis defects generally present during this period (69).

1.9.2. Late-Onset Signs and Symptoms of IEM:

In 50% of patients presenting with inborn errors of intermediary metabolism, disease onset occurs after the first year of life, during childhood, adolescence, or even adulthood. Each attack may result either in spontaneous improvement or in death despite supportive measures. Notably, the patient may be asymptomatic between two attacks. Onset of symptoms may be triggered by fever, minor infection, excessive fasting or excessive protein intake, or may occur without any apparent cause (table1.2). The clinical signs and symptoms may include neurologic findings (macrocephaly, microcephaly, attention deficit, mental and motor retardation, hypo/hypertonia, spasticity, seizures, movement disorders, stroke, ataxia, stupor, coma, psychiatric signs), muscular signs , organomegaly (hepatomegaly, splenomegaly), hematologic abnormalities (thrombocytopenia, pancytopenia), dysmorphic features (coarse facial features), skeletal abnormalities, liver failure, heart failure (cardiomyopathy), ophthalmologic findings, failure to thrive, sensory problems, and skin lesions (70)**.**

Table 1.1: Early- onset (Neonatal Period to Infancy) signs and symptoms of IEM

Main Clinical Presentation	**Presenting Sign**	**Metabolic Disease**
Neurological	Metabolic encephalopathy (coma, abnormal movements, acidosis, ketosis, hyperammonemia)	BCAA disorders (MSUD), Glutaric aciduria type II, Urea cycle defects
	Seizures +microcephaly	phosphoglycerate dehydrogenase deficiency, cerebral glucose transporter type 1 deficiency
Hepatic	Liver failure	Galactosemia ,Hereditary fructose intolerance ,Tyrosinemia type I, Mitochondrial disorders
	Jaundice	Galactosemia
	Hepatosplenomegaly	Lysosomal storage disorders
Severe hypoglycemia	Hepatomegaly	FAO defects

Table 1.1: Late-Onset the clinical signs and symptoms of IEM

Main Clinical Presentation	Other Important Signs	Metabolic Disease
Metabolic Coma	Acidosis	Organic acidurias, FAO defects ,Mitochondrial disorders
	Hypoglycemia	Gluconeogenesis ,defects Glycogen synthetase deficiency, FAO defects
Hepatic disorders	Jaundice, Encephalopathy, hypoglycaemia	FAO defects
	Hepatomegaly	Lysosomal storage diseases
Dermatology	Skin rashes	Porphyrias, Hartnup disease

1.9.3. Initial Investigations:

The initial tests to perform in a patient suspected to have a type of IEM generally consist of simple tests those could be done in majority of the medical centres, (Table1. 3) shows the most common tests. Any positive result in these tests generally helps to support the preliminary diagnosis of IEM and provides indication to perform advanced and diagnostic tests. In addition, initial therapeutic approach could be started based on these preliminary tests (71).

Table 1.2: Initial Laboratory Tests for IEM:

Samples	Test
Blood	Complete blood count, peripheral smear Blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, direct and indirect bilirubins, alpha-fetoprotein, alkaline phosphatase Electrolytes including sodium, potassium, calcium, magnesium and phosphorus Glucose, ketone body, pH Lactate, pyruvate, ammonia Prothrombin time, partial thromboplastin time, INR
Urine	Urinalysis Reducing substance in urine, thin layer chromatography for reducing substances Urine ketones Ferric chloride test Dinitrophenylhydrazine (DNPH) test Cyanide nitroprusside test Glycoseaminoglycans Keto acids

1.9.4. Advanced Investigations:

These investigations are generally performed in specialized centres for IEM and help to carry differential diagnosis further. However, these investigations are not generally diagnostic as similar or same substrates or metabolites could be increased in different IEM (72).

1.9.5. New-born Screening (NBS):

NBS is a very important act for the earliest diagnosis of patients with IEM and is of significant importance to initiate early treatment to prevent

morbidity and mortality. The first NBS effort was for PKU in 1959 and was done by Dr. Robert Guthrie. The test was a bacterial inhibition assay and has been used for NBS for many years in many countries. In the countries which implemented obligatory NBS for PKU, a significant reduction in mental retardation rate was noticed. Many countries today started doing "expanded NBS" by accurate and rapid technology (Table1.4). (73)**.**

Table 1.3: The Methodology Used for Newborn Screening for Some IEM

Amino acid disorders (general)	**Tandem mass spectrometry**
Fatty acid oxidation defects	Tandem mass spectrometry
Organic acidemias	Tandem mass spectrometry
Biotinidase deficiency	Enzyme assay

1.10. Methods of separation Amino acid in biological samples:

The development of analytical methods to measure amino acid concentrations in biological samples can contribute to research on the physiological actions of amino acids and the prediction, diagnosis and understanding of diseases. The importance of amino acids in vivo has resulted in an increasing demand for analytical methods for their determination. Most such methods use separation techniques, such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and Tandem mass spectrometry (TMS) detection (74).

1.10.1. Thin-layer chromatography (TLC):

Chromatography is the collective term for a set of separation techniques that operate based on the differential partitioning of mixture components between a mobile and a stationary phase. The mobile phase (a liquid or a gas) travels through the stationary phase (a liquid or a solid) in a defined direction.

The distribution of components between the two phases depends on adsorption, ionic interactions, diffusion, and solubility or in the case of affinity chromatography, specific interactions (75)**.** TLC works on the principle of distributing components of the mixture sample between the two phases of the technique via capillary action. TLC is operated on a glass sheet or aluminum sheet, modified with a thin layer of a granular substance such as silica gel or aluminum oxide. This granular layer acts as a solid stationary phase, which is adsorbent in nature. Further, a trace amount of sample is placed at the starting point just above the TLC plate's staring point**.** The TLC plate further is shifted to the developing chamber that contains a small puddle of solvent just beneath the level where the sample has been placed. The mobile phase (solvent) via capillary action moves up the TLC plate. As the mobile phase travels up the plate and over the sample, the components of the sample segregate according to their physical properties such as molecular structure of the compound and the type of functional group present in them. Here solubility rule is followed, that is, "like dissolves like." If the compound has more similar physical properties with mobile phase, more are the chances that it would stay in mobile phase and also the compounds that are most soluble in the mobile will be carried up the TLC plate till the farthest. On the other hand, the compounds that have more affinity for stationary phase and have low soluble rate in mobile phase will stay along with the stationary phase .In TLC, the expression of a single compound is designated by a quantitative term known as Rf value. It is calculated by the division of distance traveled by the sample from the spot position divided by the distance travelled by the mobile phase from the original site:

Rf = distance travelled by a component / distance travelled by mobile phase.

Rf value depends on various parameters such as the mobile phase used, the nature of the adsorbent, temperature, thickness of the layer, sample mass, and developing tank (76) (77)**.**

Advantages

- Simple, quick, inexpensive, high sample throughput technique
- Wide choice of mobile phases
- Minimum sample preparation
- Several samples can be run simultaneously using a small quantity of mobile phase
- Used in analytical laboratories having limited resources (78) (79).

1.10.2. *High-Performance Liquid Chromatography* (HPLC):

A liquid mobile phase could use to separate the components of a mixture. The components themselves are first dissolved in a solvent and then forced to flow (via the mobile phase) through a column (stationary phase) under high pressure. The mixture is resolved into its components within the column and the amount of resolution is dependent upon the interaction between the solute components and the column stationary phase and liquid phase the molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column. However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions (Figure1. 8) (80). Available method may be too expensive, time consuming or energy intensive, or that may not be easily automated. Existing method may come out with error, contamination prone or they may be unreliable (81).

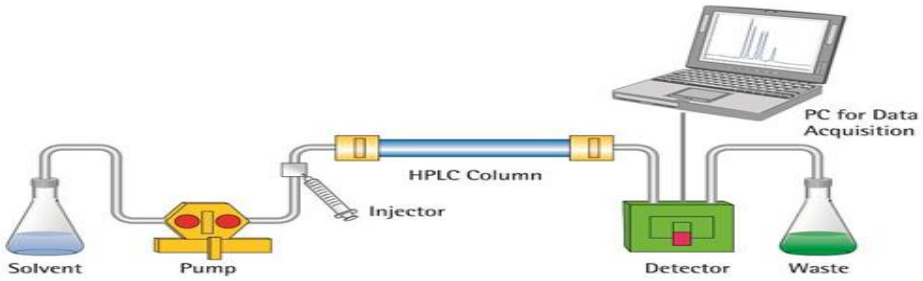


Figure 1.6 : Principle of High-Performance Liquid Chromatography (HPLC) (82).

1.10.3. Tandem Mass spectrometry (TMS):

Mass spectrometry (MS) is a high-throughput analytical detection technique used to get information about the molecular weights and chemical structures of the peptides, proteins, carbohydrates, oligonucleotides, natural products, and drug metabolites. The principle of the spectrometer depends on the separation of the molecules based on their mass-to-charge (m/z) ratio by ionization of the molecules with high energy electrons to break a molecule into fragments. Fragmentation pattern and m/z values provide information about the molecular weights and chemical structures of the peptides and proteins. Each peptide has a specific molecular weight. Molecules from solution or solid phase should be transferred into gaseous phase, because MS measurements have to be done on ionized molecules in the gaseous phase (83). MS provides some advantages including small amount of sample requirement, label free detection, fast analysis, and capability of defining chemical structures with fragmentation, high sensitivity, and simultaneous detection of multiple analytes. Characterization of a wide variety of viruses. Reliable and rapid identification Accurate and sensitive method .High throughput and Easy sample preparation. Disadvantages: Identification limited by database, Time-consuming, High cost (84).

1.11. Knowledge gap and Implications

Early undetectable and untreated IEM might lead to disability, which presents a great socioeconomic burden. Unfortunately, only few hospitals in

the Iraq including AlKadhmiya hospital has established appropriate programs for IEM disorders. More studies are needed in Iraq to monitor and study the IEM. At the public level, and since consanguinity is the main factor of having the disorder in our region, continuous awareness campaigns through media, schools, and universities are recommended to educate the public about potential health risks posed by marriage between close relatives. Genetic counsellors also play a big role in educating and helping the parents and affected siblings in not having another affected child during future pregnancy by introducing them to primary prevention such as prenatal diagnosis or Preimplantation Genetic Diagnosis (PGD). Issuing a policy through governments to mandate the screening test for every new born is one effective approach to reduce IEM. Due to the rarity of specialized experts in this field, physicians, scientist, lab technologist, and governments should support training programs to compensate for this inadequacy.

1.12. Aims and Objectives of the study:

- To review the background documents on the state of the art of the scientific literature in this area of work.
- To examine the feasibility of employing thin layer chromatography for qualitative analysis of amino acid.
- To suggest areas where further research is needed, either to deal with gaps in the knowledge related to develop and diagnose the disorders of amino acid metabolism.
- This study was also aimed to introduce a separation method of standard amino acid that could be effective for the detection amino acid disorders by producing distinguishable strategies based on silica gel TLC plates.

Chapter Two

Materials

And

Methods

2. Methodology

2.1. Study design

The present work included a cross section study from September 2020 till January, 2021. Samples were selected from the patients attending the rare diseases unit, Kerbala teaching hospital for children. The sociodemographic aspects of the patients were collected through the self-reported technique (student questionnaire) including age, gender, Genetics History of family, congenital abnormalities and having any current chronic diseases. They were also exposed to medical examination for signs and symptoms of IEM by sub-specialized doctor based on the World Health Organization (WHO) criteria.

2.2. Materials and Methods:

2.2.1. Chemicals

In this chapter, chemical, instruments and tools were listed in Table 2-1.

Table 2-1 Chemical used in current study

No.	Chemicals	Source (Country)
1.	Thin layer chromatography plate (TLC)	Lot: 812347 Macherey-nagel(Germany)
2.	20 Amino acids	Sigma –USA
3.	Ninhydrin	Thomas baker (India)
4.	Mobile phase(butanol ,acetic acid, acetone and distal water)	Sigma /USA
5.	Ethanol	Scharlau (Spain)
6.	Chloroform (stabilized with amylene)	Sigma –USA

Table 2-2 Instruments and apparatus used in current study.

NO	Apparatus	Manufactures
1.	Electronic balancer sensitive	Radwag (Poland)
2.	Electric shaker	Bioneer (England)
3.	Centrifuge	Human Humapette (Germany)
4.	Developing chambers	China
5.	Freezer	Cooltech(China)
6.	Dryer	Enzo Professional(Italian)
7.	Micro Pipette(100-1000µl)	Human Humapette (Germany)
8.	Micro pipette(10-100 µl)	Human Humapette (Germany)
9.	Gilson Tips,1000µl (blue)	China Mheco(China)
10.	Gilson Micro-tips, 100µl (yellow)	China Mheco (China)
11.	Anticoagulant tube	Lot:1912 China Mheco(China)
12.	Glass test tube	Lot:20160825 China Mheco(China)
13.	Capillary tube (blue)	Lot :1910436 China Mheco(China)
14.	Pencil	
15.	Ruler	

2.3. Study groups

The study were included 56 samples, ages ranging from 7 day to 15 years. The patient samples were divided as follows: Neonate of age < 1month were

seven, new born aged from 2-12 months numbered 39, children aged >1year were 10, 34 male and 22 female of them suspected inborn errors of metabolism .The study protocol was approved by the ethical research committee, College of Medicine, University of Karbala and Karbala Health Directorate.

2.4. Patient Criteria:

Patients were selected from Kerbala teaching hospital for children, This study consists of 56 patients suspected with inborn errors of metabolism which were selected from the rare diseases unit, in Karbala governorate. Samples size was calculated based on the prevalence of IEM .Weight, height, signs and symptoms, and the relationship between parents were recorded. A questionnaire was designed in order to identify important sociodemographic characteristics, general health status, family history of chronic diseases, and dietary habits.

2.5. Approval of ethical:

The protocol of the study was approved by Ethical Committee of Karbala Medical College, and committee of the rare diseases unit, Kerbala teaching hospital for children .Samples (serum and urine) were obtained after consent from patients or the patients' relatives.

2.6. Samples collection:

2.6.1. Blood collection and storage:

For all participants 3 ml of blood was taken from the vein by sterile syringe and transported in gel tube container to the central lab in Kerbala teaching hospital for children. And then centrifuged for 3 minutes at 4000 x g to separate serum. Serum then transported by micropipette e to sterile

container. Serum samples were stored and saved at 10°C until analyses. Blood collection tubes were be disposable, non-pyrogenic, and non-endotoxin.

2.6.2. Urine collection and storage

Three ml of urine samples were collected from patients by giving the parents a disposable urine cup, and urine samples were stored and kept in refrigerator for the working day.

2.7. Methods:

2.7.1. Preparation Standard Amino acid:

Standardization of AA by TLC was performed for the standard. 0.1g of each stock amino acid was dissolved in 1ml distal water and spotted in TLC, the concentration of AA were listed in the table below:

No	Amino acid	Abbreviation	Molecular weight g/mole	Concentration Molarity
Nonpolar ,aliphatic R-group				
1	Glycine	Gly	75	1.3 M
2	Alanine	Ala	89	1.1 M
3	Proline	Pro	115	0.86 M
4	Valine	Val	117	0.85 M
5	Leucine	Leu	131	0.76 M
6	Isoleucine	Lle	131	0.76 M
7	Methionine	Met	149	0.67M
Aromatic R-group				
8	phenylalanine	Phe	165	0.60 M
9	Tyrosine	Tyr	181	0.55 M
10	Tryptophan	Trp	204	0.490 M
Polar , uncharged R –group				
11	Serine	Ser	105	0.95 M
12	Threonine	Thr	119	0.84 M
13	Cysteine	Cys	121	0.82 M

14	Aspargine	Asn	132	0.75 M
15	Glutamine	Gln	146	0.68 M
Positively charged R-group (basic amino acids)				
16	Lysine	Lys	146	0.68 M
17	Argnine	Arg	174	0.57 M
18	Histidine	His	155	0.64 M
Negatively charged R- group (acidic amino acids)				
19	Aspartate	Asp	133	0.75 M
20	Glutamate	Glu	147	0.68 M

2.7.2. Preparation of mobile phase for (TLC):

Fourteen ml of butanol (99.8 %) +14ml of acetone (99.9 %) +4ml of Acetic acid +8ml of distal water.

2.7.3. Preparation of Ninhydrin reagent:

Ninhydrin solution was prepared by dissolving 0.02 g of Ninhydrin in 10 ml of ethanol (95%).

2.7.4. Preparation of serum patient's samples:

Serums were isolated soon after collecting by centrifuging for 3 minutes at approximately 4000×g, serum samples were stored at refrigerator, then 0.5 ml of the serum was dissolved in 1 ml of ethanol, shaked and centrifuged for three minutes at approximately 4 000 ×g. Then 250 uL of the supernatant was transfer to the can tube to the time of analysis.

2.7.5. Preparation of urine patient's samples:

Urine samples were collected from the patients. Each 1 ml urine samples were mixed with 3 ml of ethanol, shaked, and then centrifuged for three minutes at approximately 4 000 ×g. After that only 1 ml of the mixer supernatant was separated and mixed with 3 ml of chloroform. This mixture was placed in a glass can tube, shaked and centrifuged for three minutes at

approximately 3 000 ×g. Only the oily layer was separated then placed in a can tube to the time of analysis.

2.7.6. Preparation of stationary phase for (TLC):

The silica gel 60F 254 precoated thin-layer chromatography (TLC) plates (20 cm×20 cm, 0.20 mm) from Macherey-nagel (Germany) were used for amino acids analysis. The solutions (standard amino acids and patient samples) were applied to the points marked with a pencil on a line at equal distances where the points are within one centimeter of the edge of the plate. All stains were dried with a dryer prior placing the plate in the glass chamber.

2.7.7. Principle of the TLC Assay:

With an anti-coagulant capillary (test tube blue), three spots were applied from dissolved standard amino acids, While 5 drops of pre-prepared patient samples are applied and placed on the line drawn (1 cm from the edge) of the thin layer. Taking into account the size of the drop and the time period between drying of each drop and the other. The prepared plate with sample spotting was placed into the glass chamber (A standard TLC glass chamber rectangular TLC developing tank complete from Aldrich; 25 cm x 23.5 cm x 5.0 cm; L x H x W) as evenly as possible and lean against the side of the plate, Never allow the bulk solvent to rise above the start line, Then the chamber was closed with a lid. Allow capillary action to draw the solvent up the plate and sufficient time (one hour) was given for the development of spots. Then, the plate was removed and immediately end labeled across the solvent front then, allowed to dry with dryer. After that, it was sprayed with ninhydrin (0.01M) which was prepared by dissolving 0.02 g in 10 ml of ethanol (95%) .

2.7.8. Calculate the retention factor for each amino acid:

Every amino acid appears as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent. The Rf formula is

$$RF=\frac{Distance\ traveled\ by\ solute}{Distance\ traveled\ by\ solvent}$$

2.8. Statistical analysis:

Data analysis for this work was generated using the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copyright (2013– 2020) (85).

Information from the questionnaire and all test results from patients and control samples were entered in data sheet. Descriptive statistics were performed on the participants' data of each group using different Advanced excel charts such as tornado chart which was used to show the compression of different IEM complication among patients group.

Chapter Three

Result and discussion

3. Results and Discussion

3.1. Demographic and clinical characteristics:

Inborn errors of metabolism (IEM) are a group of over 500 heterogeneous disorders resulting from a defect in functioning of an intermediate metabolic pathway. Although outcomes can often be, good if recognised early. Due to the significantly contribution of IEM to the burden of non-communicable childhood morbidity, this study was conducted to examine the feasibility of employed Thin Layer Chromatography for qualitative analysis of amino acid related to develop and diagnose the disorders of amino acid metabolism.

The clinical demographic characteristics and laboratory parameters of patients groups were summarized in Table (3.1).

The study were included 56 patients referred with explained symptoms with the suspicion of inborn error of metabolism, patients number was calculated based on the sample size equation. Patients referred to different general and private paediatric hospitals and clinics. The current work illustrate the mean age of participants which was within ages ranging from 7 day to 15 years. Infants of age < 1month were7, newborn aged from 2-12 months numbered 39and children aged >1year were 10. Regarding the gender, the number of females was 22 and males represent 34. Patients with positive consanguinity were 49 (87%). The patients who had a strong positive family history were about 33 (52%).

Table 3-1 Descriptive of the Demographic and laboratory characteristics of the study population.

Sex		Congenital abnormalities No.		Mode of delivery No.		Father smoker No.		Intellectual delay No.		Ophthalmological problems No.	
Male No.	Female No.	With	Without	Caesarean birth	Vaginal birth	Yes	No	with	Without	with	Without
34	22	5	51	36	20	42	14	22	34	3	53

Since the parental consanguinity is the main cause of the inherit age IEM, results were shown a highly association between them. The relationship between father and mother as a risk factor of IEM was 87% while the effect of family history was 52% and only 25% was for the history of effecting sibling as shown in (Figure3.1). This association with consanguinity predictable since all cases of IEM have an autosomal recessive mode of inheritance, this meaning family members may be carriers and not affected by the disease. Absence of the disease in family members does not rule out IEMs (86). In the study, consanguineous unions were found to be more frequent among people who were belonged to tribes with a high frequency of consanguineous marriages. However, there was limited data about this rate in Karbala. But, in general, a high rate of consanguinity was recorded in Iraq reaching an average of 47–60%, which may be related to the social, cultural, religious, and political factors (87).

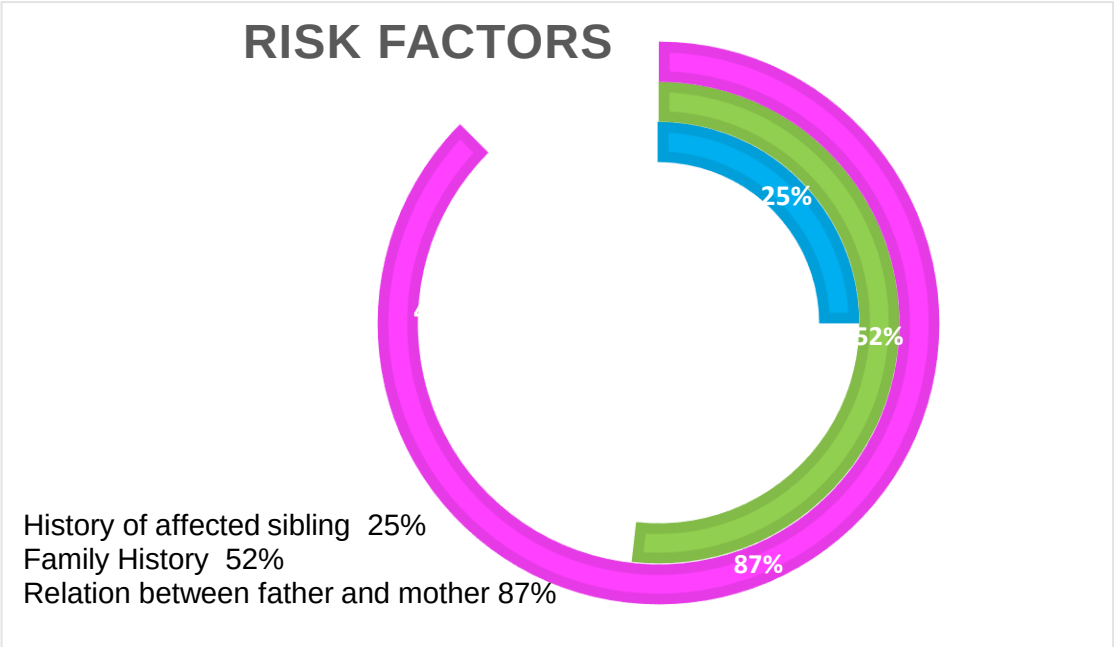


Figure 3-1 Local risk factors of inborn error of metabolism in Karbala/ Iraq

3.2. Study of complications of inborn errors of metabolism

The complications of inborn error of metabolism in this study were divided to four groups: complications of Gastrointestinal Tract, neurological Complications, Chief Complains, and other rare complications. Our study during shows (Figure 3.2) demonstrated the number of patients who were with or without the complications of Gastrointestinal Tract. Figure shown that 41% of the suspected IEM patients were with sign of jaundice (pale face and sclera), and 32%, 29% ,18%,16% ,5%, 5% with flatulence, vomiting , diarrhea , acidosis, dehydration and hepatomegaly respectively.

Many studies were reported that the best-known metabolic disease associated with jaundice is galactosemia, in which deficiency of the enzyme galactose-1-phosphate uridyl transferase results in accumulation of galactose-1- phosphate and other metabolites such as galactitol that are thought to have a direct toxic effect on the liver and other organs.

Other were reported that vomiting is characteristic as a feature for many groups of IEM because ammonia is ineffectively eliminated by the kidneys

and the body tries to excrete urea through Digestive (88). On the other hand, inborn errors of metabolism with acidosis could be result from the Organic acidemias and defects of pyruvate and ketone body metabolism (89). While hepatomegaly was consider as a common clinical finding in several forms of storage disorders (Glycogen storage disorders and Lysosomal storage disorders). It can be either an early or only sign of a hepatic manifestation of an inborn error of storage metabolism (90). Lactose intolerance also consider as a clinical syndrome of one or more of the following: abdominal pain, diarrhea, nausea, flatulence, and/or bloating after the ingestion of lactose or lactose containing food substances (91).

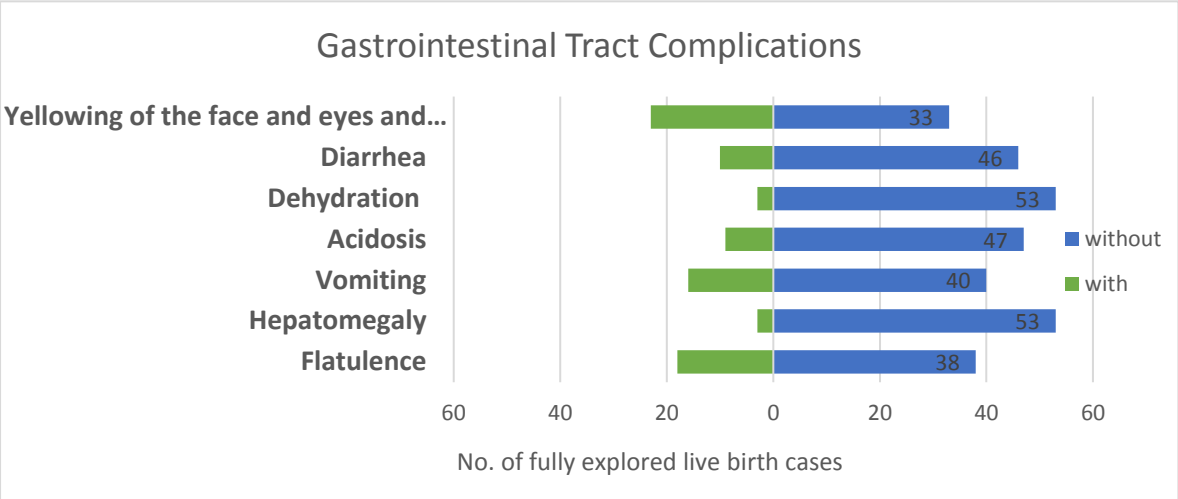


Figure 3-2 the number of patients who were with/without Gastrointestinal Tract Complications in IEM patients.

Neurologically the most common manifestations of IEM in the neonatal period are decreased level of consciousness; seizures; hypo- or hypertonia.

Results were shown about 39%, 18%, 41%, 50%, 36% of suspected IEM patients were with Intellectual delay, Lethargy, Spasticity, Hypotonia, Epileptic seizures respectively (see (Figure3.3).

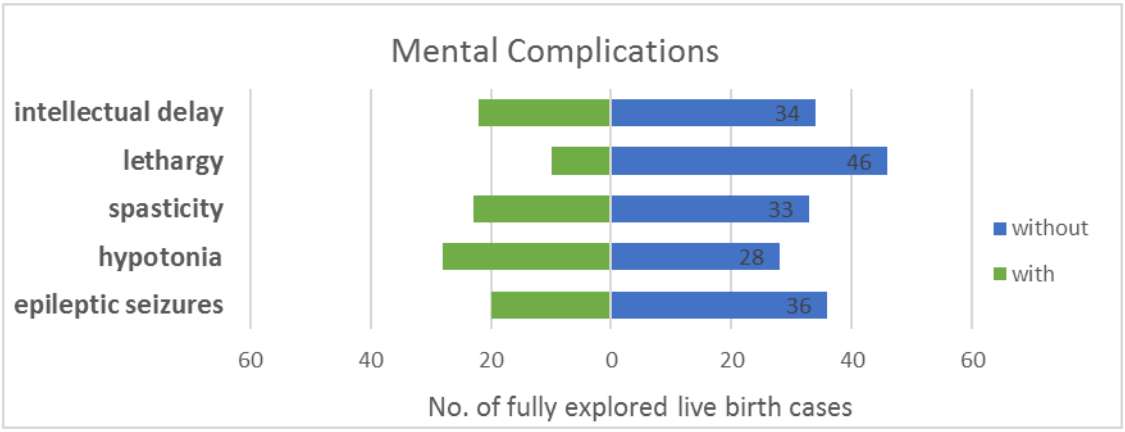


Figure 3-3 the number of patients who were with neurological complications in IEM patients.

Coma in a neonate is likely due to an intoxication metabolic disorder such as UCD and OA and often treatable. Hypotonia as the only presentation in a neonate caused by an IEM is rarely treatable (92) (93). Also, many IEMs can present with severe hypotonia, muscle weakness and poor muscle mass can be seen in urea cycle disorders (UCD) and organic acidemias. Severe neonatal generalized hypotonia and progressive myopathy can be seen in primary and secondary mitochondrial disorders, fatty acid oxidation defects (FAO), peroxisome biogenesis disorders (PBD), glycogen storage diseases (GSD), and some other lysosomal storage diseases (94). Mental status changes in a new-born can be seen in several underlying common biochemical derangements, such as metabolic acidosis, hyperammonemia, or hypoglycaemia (95). The alteration in mentation may progress from irritability to lethargy and ultimately a comatose state. In general, coma due to metabolic derangements is associated with abnormal movements of the limbs or elevated muscle tone, or neuro-vegetative symptoms such as hiccups. A comatose state associated with hypotonia is more commonly caused by non-metabolic processes but may also be seen in IEM such as UCDs (96). On the other hand, IEM that lead to energy deficiency may show a progressively worsening clinical course without initial symptom-free interval and with more variable

severity of initial presentation. These IEM are also more likely to be associated with cardiac or hepatic abnormalities. Seizures in the neonatal period are another common initial finding of IEM, and unexplained or intractable seizures in a neonate should always raise the suspicion for IEM (97). Seizures may present with a mixed semiology including partial, generalized, myoclonic, and tonic seizures (98).

Chief Complains of IEM varies with age at study presentation based on which various inborn errors of metabolism (IEMs) manifest clinically. Symptoms could range from abrupt in onset and episodic to chronic and progressive. Results indicated that the percentage of the chief complains mainly were 20% for Jaundice and 16% for delayed growth, 10% for both lethargy and vomiting as shown in (Figure3.4).

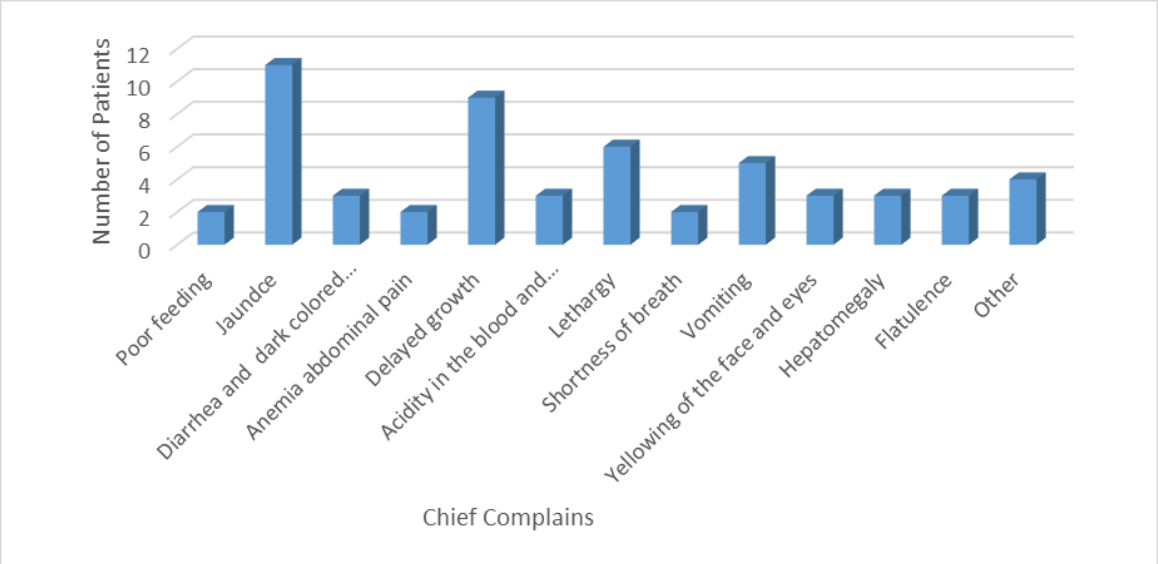


Figure 3-4The number of patients who were with Chief Complaint in IEM patients.

galactosemia usually causes no symptoms at birth, but jaundice, diarrhea, vomiting, and cataract formation soon develop and the baby fails to gain weight. hypoglycemia may be observed. The disease may present initially with indirect hyperbilirubinemia resulting from hemolysis secondary to high levels of galactose-1-phosphate in erythrocytes. In addition, the effects of acute galactose toxicity in the brain may sometimes because the CNS symptoms to

pre- dominate. Escherichia coli infections are common in untreated galactosemic infants, and death can occur at as early as 1 to 2 weeks of age from severe E coli infections. The American Liver Foundation recommends that all infants who develop jaundice be considered for galactosemia (99). Furthermore, vomiting also was one of the inborn errors of metabolism and might be consider as one of the patients differentiation presenting with a cyclic vomiting syndrome phenotype. Classes of disorders include: mitochondrial disorders, fatty acid oxidation disorders, urea cycle defects, organic acidurias, and acute intermittent porphyria. Children with cyclic vomiting syndrome manifest with periodic attacks of nausea with frequent vomiting. Attacks can last from hours to days. Typically, attacks have a certain periodicity and are stereotyped within an individual (100). Onset is most commonly around age five years (101). Many previous studies have been reported that galactosemia is the main reason of the long-term complications of IEM (102) (103) (104) (105)**.** These appear to be independent on the severity of initial illness and the strictness of dietary compliance, growth retardation, decreased bone density, verbal dyspraxia, difficulties in spatial orientation and visual perception, and intellectual deficits have been variably described as complications of galactosemia (106). Commonly, the organic acidemias, such as glutaricacidemia type II, pyruvate carboxylase deficiency, or urea cycle disorders has been reported to result in Hyperammonemia, along with respiratory distress and metabolic acidosis (107). (Figure 3.5) demonstrated the rare complication of the suspected IEM.

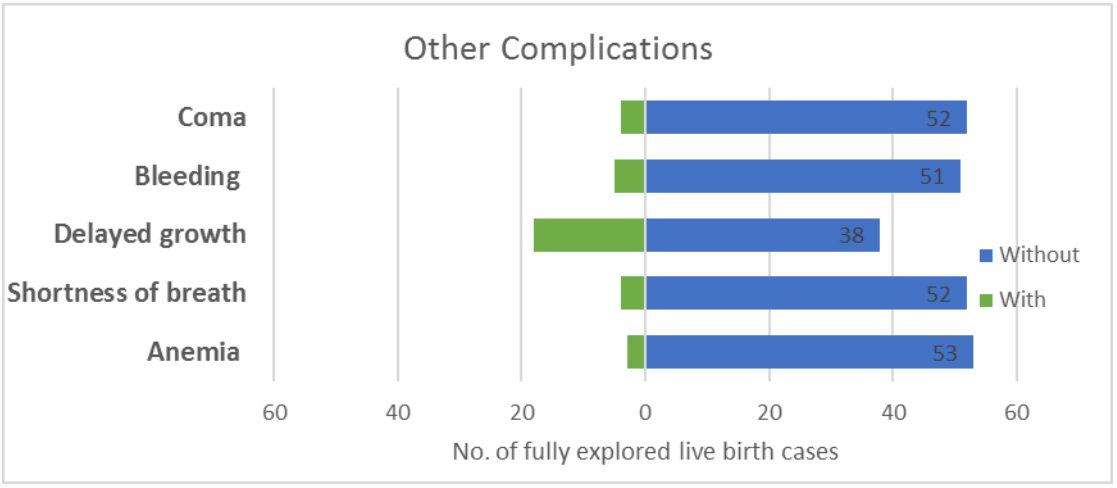


Figure 3-5 the number of patients who were with other complications in IEM patients.

Study was also including examination the distinctive cranial development. Previously, it was reported that about 60% of infants develop microcephaly due to Glucose Transporter Defect (GLUT1 Deficiency Syndrome) (108). Current study indicated that 32% of the patients had Microcephaly while 27% were Macrocephaly and 41% were Normal as shown in (Figure3.6)

Short-chain acyl-CoA dehydrogenase deficiency (SCADD), a mitochondrial fatty acid metabolism disorder, might results in an accumulation of butyrylcarnitine and ethyl malonic acid in blood and urine which could cause microcephaly (109).Glutaric aciduria type I(GA I) caused by the deficiency of glutaryl CoA dehydrogenase, a mitochondrial matrix enzyme involved in the degradation of lysine, hydroxyl lysine, and tryptophan, Macrocephaly may be the first manifestation of GA- I (110).

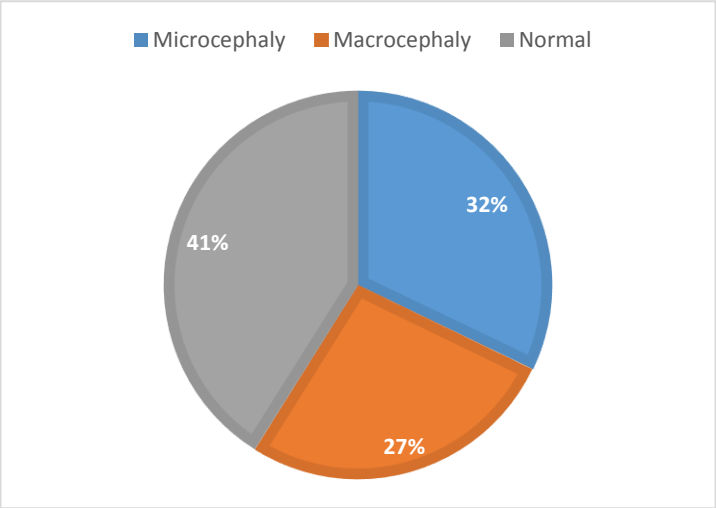


Figure 3-6 the number of patients who were with abnormal cranial development in IEM patients.

Effect the types of feeding were also investigated thoroughly, since disease is a genetic disorder; most new-borns are born naturally at birth (free of any symptoms of the disease). Disease symptoms start to appear immediately after feeding. When the milk is catabolised, it produces a complex proteins, carbohydrates and fats, and these substances are broken down into (amino acids, glucose and fatty acids). Therefore, any genetic defect in any enzyme responsible for catabolizing these substances (amino acids, glucose, and fatty acids) may showing symptoms of the disease.

(Figure 3.7) showing that using both natural and artificial milk could be a factor in the emergence of symptoms of the disease. Sometimes the symptoms may be delayed for months after childbirth, and the reason is a partial defect in the work of the enzyme which leads to accumulation of those substances (amino acids, glucose, and fatty acids) inside the body's organs (such as the brain) which resulting from the breakdown of milk. Therefore the early diagnosis could be a key factor for direction a special medical treatment protocol (111).

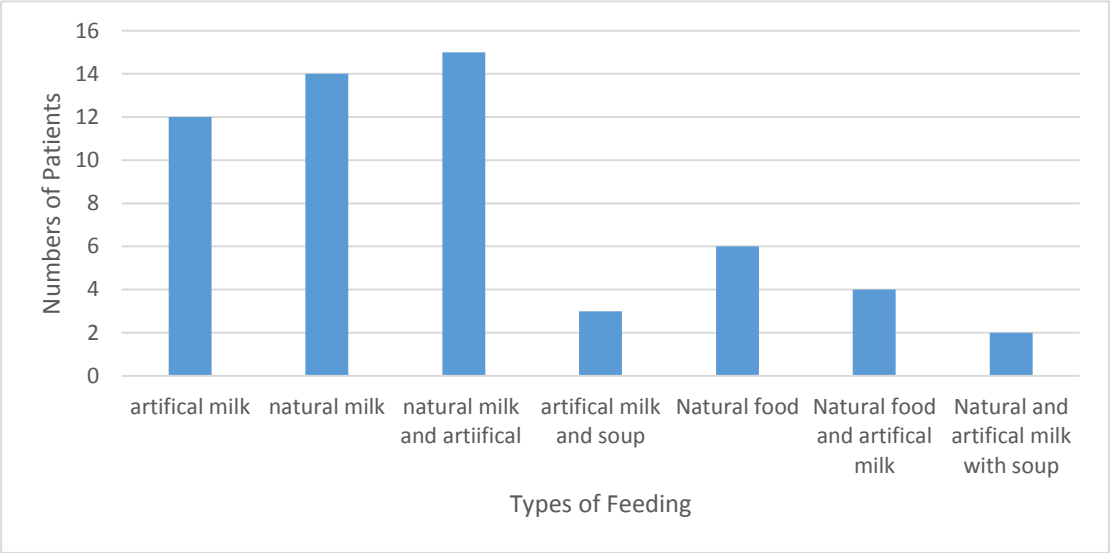


Figure 3-7 Distribution of suspected IEM patients based on types of feeding

3.3. Examination the standard amino acid by TLC

This study was attempted to examine the basic ideas and the significance of Thin layer Chromatography (TLC) as laboratory methods used for preliminary method prior to mass spectrophotometry. This could be performed with less complicated technique to analysis amino acid disorder in patients with IEM.

It has performed precisely the 20 amino acids and separated them according to different ways which were listed below:

No	Separation principle
1	Depending on the arithmetic mean of the retention factor
2	Depending on the range (minimum - maximum) retention factor of ten readings
3	Based on amino acid colour on TLC plates with ninhydrin spray reagent
4	Based on the shape of the amino acid spot on TLC
5	according to the distance travelled by sample of each amino acid for ten readings

3.3.1. Standardisation the AA by TLC based on the arithmetic retention factor

All 20 amino acids were standard by TLC on a regular silica plate based on arithmetic retention factor. The retention factor (R*f*) values could be reflect the behavior of an individual AA in TLC, which is characterized by a quantity Known as R*f* and is expressed as a decimal fraction. The R*f* is calculated by dividing the distance the compound traveled from the original position by the distance the solvent travelled from the original position (the solvent front), generally, It is a constant for each component only under identical experimental condition.The calculation of Rf for each amino acids were performed by ten repeated runs. Rf values was calculated and compared with the repeated values to identify the amino acids. The Rf value for each known compound should remain the same provided the development of plate is done with the same solvent, type of TLC plates, method of spotting and in exactly the same conditions.Samples of Tyrosine and Phenylalanine amino acid standard runs were shown in Figures (3.8) (3.9). The figures illustrated the ten Rf reading, and the rest of amino acid were shown in the appendix (figures 20).

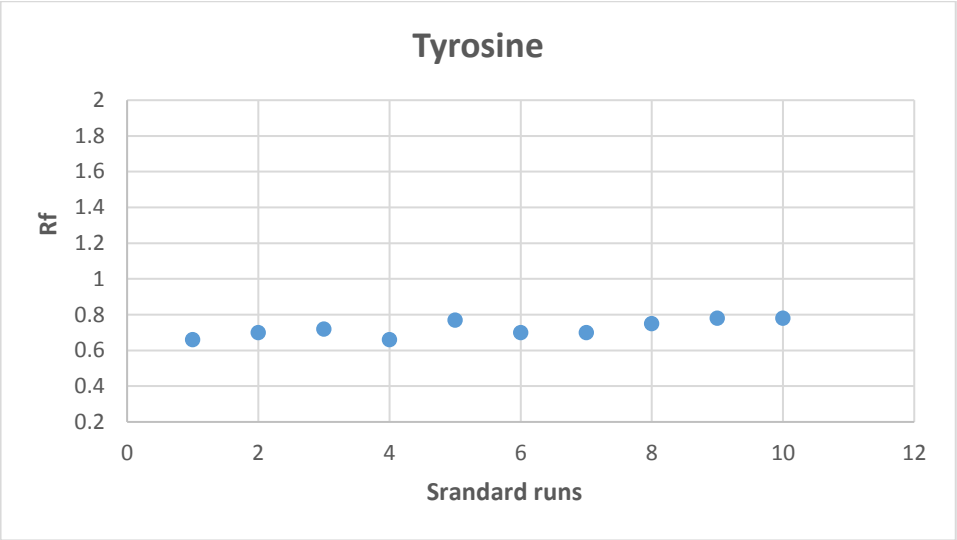


Figure 3-8 Calculated Rf of standard amino acid (Tyrosine) from ten repeated runs by TLC.

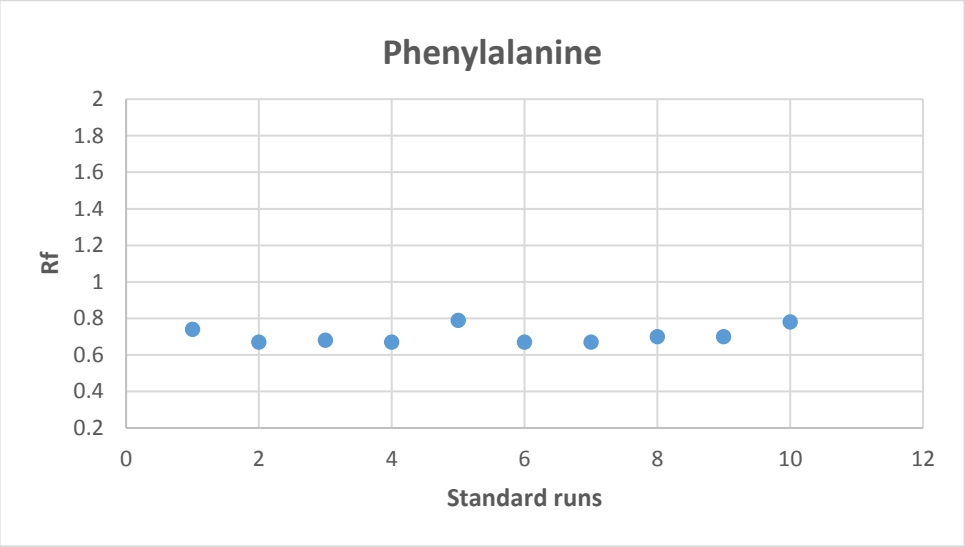


Figure 3-9 Calculated Rf of standard amino acid (Phenylalanine) from ten repeated runs by TLC.

On the other hand, the Amino acids were divided into six subgroups according to the rounding Mean of Rf for each amino acid in an attempt to make the identification of the amino acid more and easily separated since the preparative TLC can be a far more efficient in terms of time and cost than doing mass spectrophotometry. The groups of AA were listed in the Figures below. Group A was included the amino acids (Glycine, Serine , Histidine , Alanine , Aspargine) the range of their Rf was (0.4, 0.42, 0.42, 0.43, 0.43) respectively

as demonstrated in (Figure 3.10) , Group B was included the amino acids (Threonine, Glutamic, Valine, Cysteine) the range of their Rf was (0.49, 0.51, 0.54, 0.55) respectively as demonstrated in (Figure 3.11), Group C was included the amino acids (Proline, Glutamine, Aspartic) the range of their Rf was (0.46) as demonstrated in (figure 3.12), Group D was included the amino acids (Methionine, Isoleucine, Leucine) the range of their Rf was (0.66, 0.68, 0.69) respectively as demonstrated in (Figure 3.13), Group E was included the amino acids (Phenylalanine, Tyrosine , Tryptophan) the range of their Rf was (0.7, 0.72, 0.73) respectively as demonstrated in (Figure 3.14), while Group F was included the amino acids (Lysine, Arginine) the range of their Rf was (0.19, 0.33) as demonstrated in (Figure3.15).

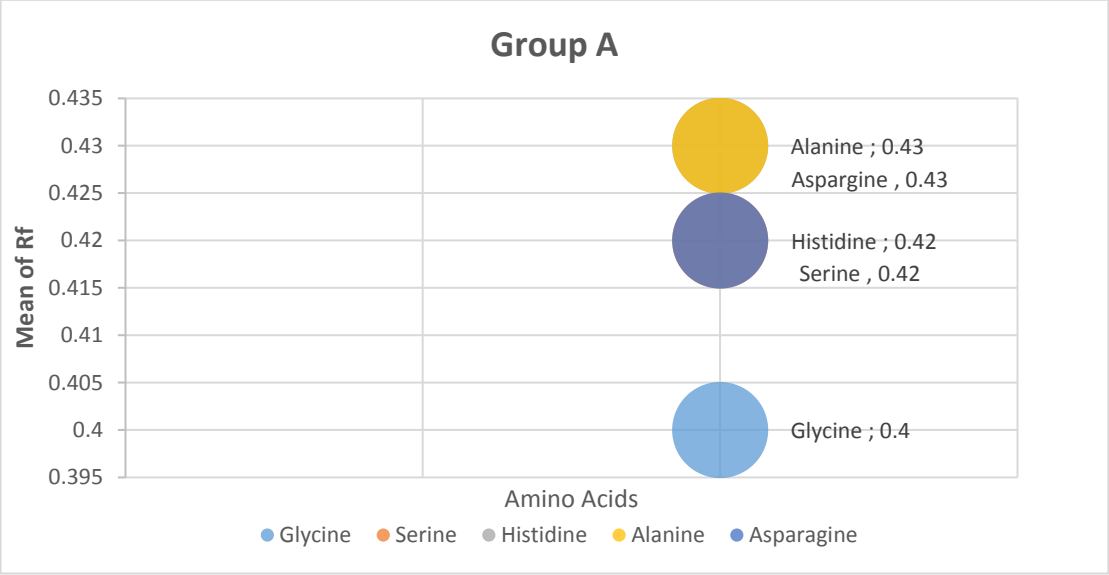


Figure 3-10 The mean of retention factor (Rf) values of standard Amino acids (Group A) including (Glycine, Serine , Histidine , Alanine , Asparagine) separated by TLC

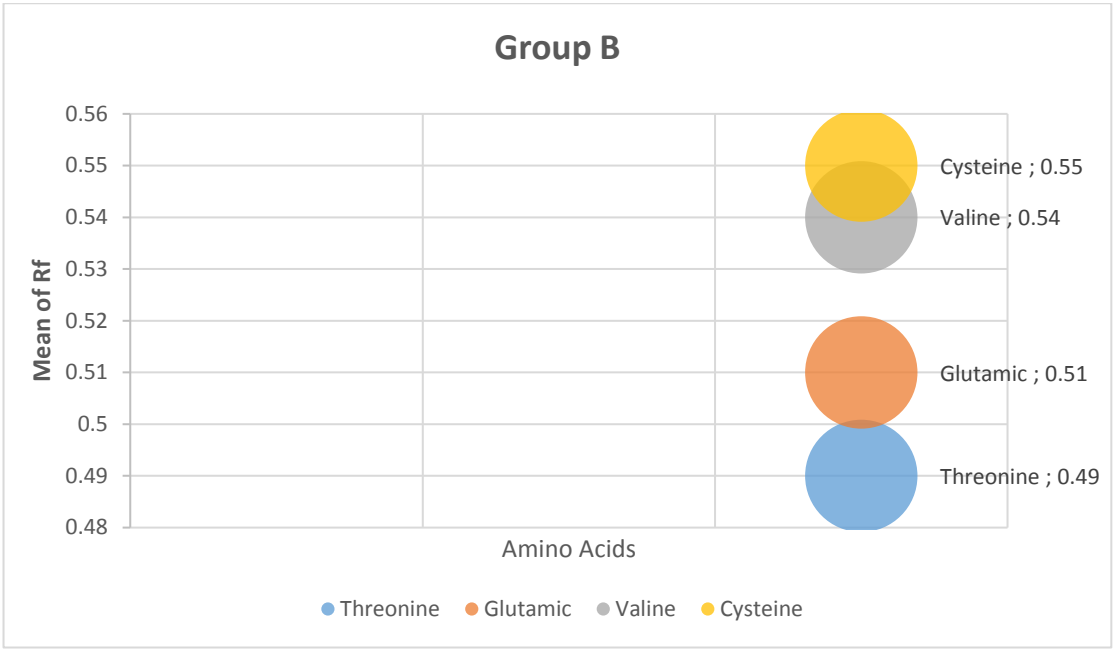


Figure 3-11 The mean of retention factor (Rf) values of standard Amino acids (Group B) including (Threonine, Glutamic, Valine, Cysteine) Separated by TLC

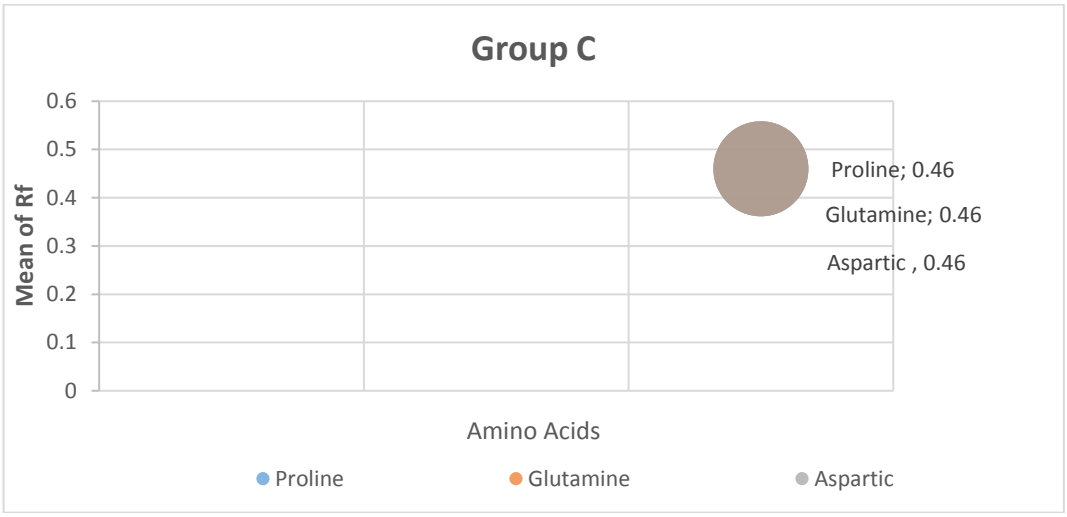


Figure 3-12 The mean of retention factor (Rf) values of standard Amino acids (Group C) including (Proline, Glutamine, Aspartic) separated by TLC

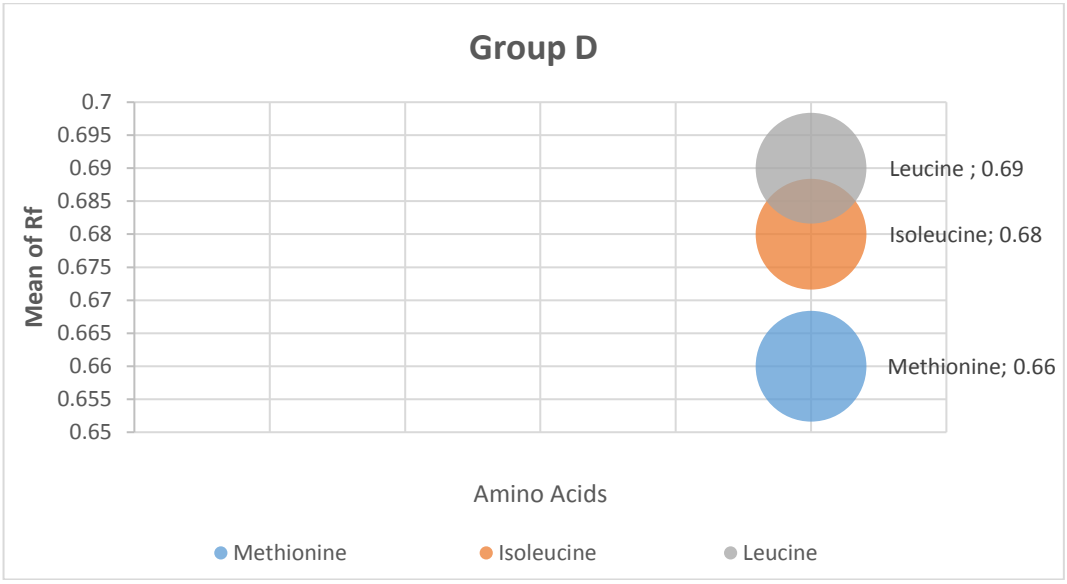


Figure 3-13 the mean of retention factor (Rf) values of standard Amino acids (Group D) including (Methionine, Isoleucine, and Leucine) separated by TLC

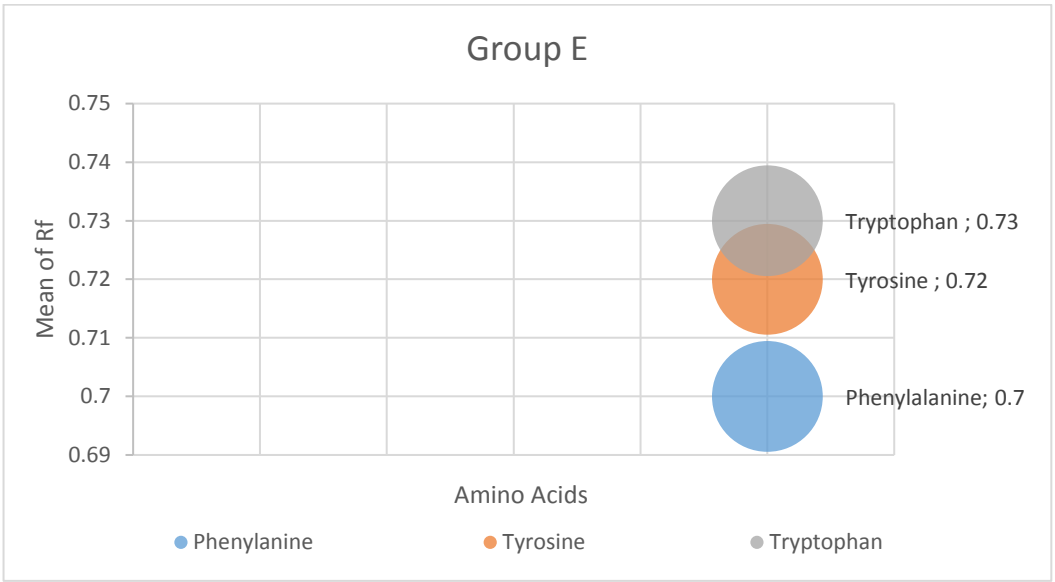


Figure 3-14 the mean of retention factor (Rf) values of standard Amino acids (Group E) including (Phenylalanine, Tyrosine, and Tryptophan) Separated by TLC

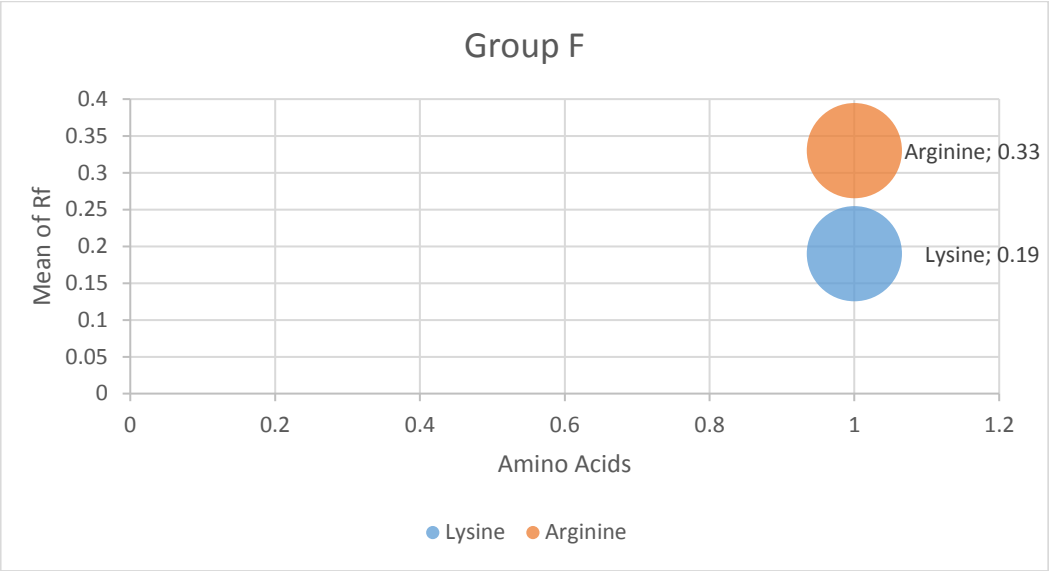


Figure 3-15 the mean of retention factor (Rf) values of standard Amino acids (Group F) including (Lysine, Arginine) separated by TLC

3.3.2. Standardisation the AA by TLC depending on the range (minimum - maximum) retention factor

Since the different AA were shown a similarity in the mean of Rf due to their traveling in a same rates because of their attraction to the stationary phase and/or their solubility in the solvent, therefore another Standardisation were done depends on the range (minimum - maximum) of the retention factor for ten repeated readings. The range of Rf of each AA were listed in the (Table 3-2).

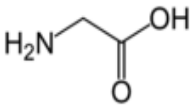
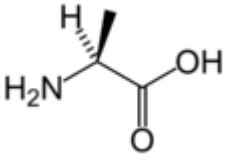
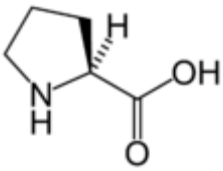
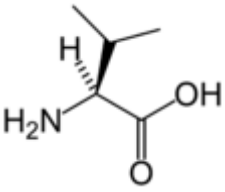
Table3-2 Range of the retention factor for the 20 Amino acids

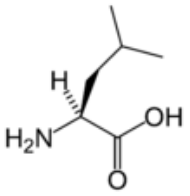
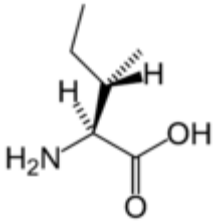
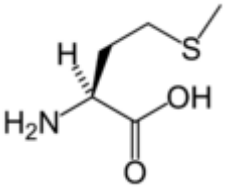
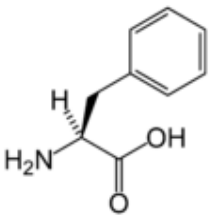
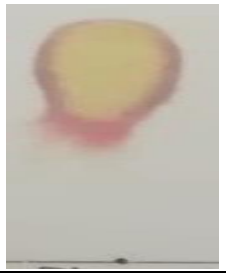
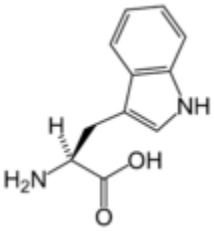
No	Amino acids	Range of RF	N0	Amino acids	Range of RF
1	Glycine	0.34-0.50	**11**	Serine	0.40-0.48
2	Alanine	0.37-0.49	**12**	Threonine	0.45-0.55
3	Proline	0.38-0.61	**13**	Cysteine	0.50-0.63
4	Valine	0.53-0.59	**14**	Asparagine	0.41-0.48
5	Leucine	0.65-0.79	**15**	Glutamine	0.42-0.52
6	Isoleucine	0.62-0.72	**16**	Lysine	0.13-26
7	Methionine	0.62-0.76	**17**	Arginine	0.30-0.36
8	Phenylalanine	0.67-0.79	**18**	Histidine	0.39-0.48
9	Tyrosine	0.66-0.78	**19**	Aspartic	0.43-0.50
10	Tryptophan	0.65 -0.79	**20**	Glutamic	0.50-0.55

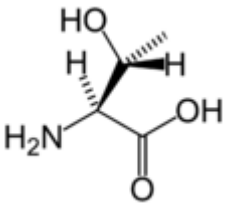
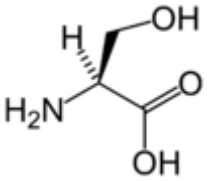
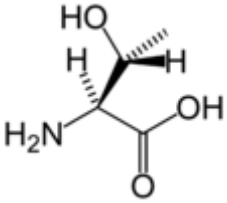
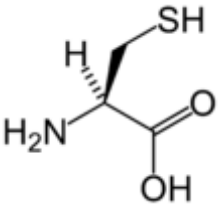
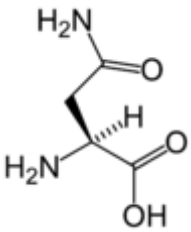
3.3.3. Standardisation the AA by TLC based on amino acid colour in the TLC plates with ninhydrin spray reagent

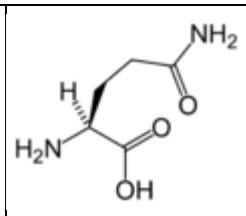
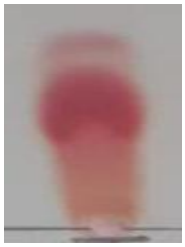
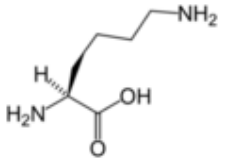
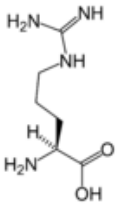
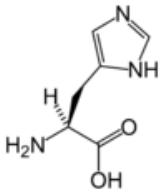
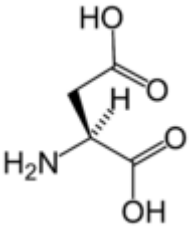
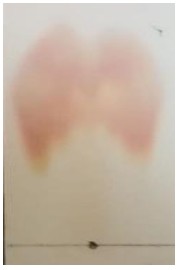
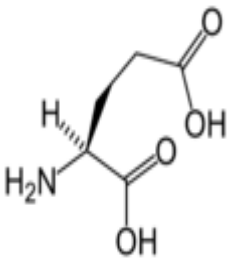
Apparently, some amino acids were impossible to distinguish by their Rf due to showing a sort of interference, therefore a new suggested way was performed in order to get a more appropriate separation of the Standard solutions of amino acids by examine the differences of the amino acid colours and shapes in the TLC plates with ninhydrin spray reagent. Since amino acids are colourless compounds, ninhydrin is used for detecting them. Ninhydrin reacts with α- amino acids that results in range coloured spots. Colours were observed visually, and the colour pictures of chromatograms by digital camera were recorded. (Table3-3) was illustrated the colour of the 20 standard amino acid.

Table 3-3 Colour formation of amino acids on TLC plates with ninhydrin reagent

Amino acid	The chemical composition of amino acids	Shape Observation	
Nonpolar ,aliphatic R-group			
Glycine			elongated oval shape with Yellowish brown colour
Alanine			Fingur print shape with deep raddish
Proline			Circle shape lemon
Valine			Candel shape Reddish pink

Leucine			Candel shape Reddish brown
Isoleucine			Ice cream shape Reddish cone with brown cup
Methionine			Chef hat shape Pinkish brown
Aromatic R-group			
Phenylalanine			Circle Cloud shape Yellowish violet
Tyrosine			Fog Light yellowish pink

Tryptophan			Smoke shape Brownish violet
Polar , uncharged R –group			
Serine			Fingure print shape Deep pink
Threonine			Cylinder shape Bluish violet
Cysteine			Rocket shape Pale cream
Aspargine			M shape Light yellowish brown

Glutamine			Clock shape Orangish pink
Positively charged R-group (basic amino acids)			
Lysine			Candel flame shape Yellowish pink
Argnine			Tooth shape Purple
Histidine			elongated oval shape light pink
Negatively charged R- group (acidic amino acids)			
Aspartate			Lungs Yellowish pink
Glutamate			Tooth Yellowish viloet

The newly designed Standardization of AA which was established to detect the twenty amino acids on thin-layer chromatography plates based on different strategies shown good demonstration throughout the Rf and producing various distinguishable colours.

Although TLC can be used easily for routine analysis, this chromatographic system could be involved in a combination ways which might be results in better performance.

It can be concluded that based on the results obtained from the simple fingerprint TLC analysis will be helpful in identification and standardization of AA and can be utilized as a reference for the identification of AA disorder such as in IEM.

3.4. Clinical application of Standardization Amino Acid by TLC

Generally, Physicians in the public health service who wished to request any screening test for suspected IEM patients have to send it for outpatient's clinic because no higher performance methods are available locally. Definitive laboratory diagnosis is crucial in confirming clinical suspicion of AA disorder/ IEM since its clinical presentation is non-specific and can mimic common conditions.

Early diagnosis by the expanded newborn screening programme using Tandem Mass Spectrometry (TMS) in the developed countries followed by early intervention during the presymptomatic or early symptomatic period have been shown to improve the outcome of patients with IEM which is available only outside and generally sending to the neighbour country (Jorden). Delayed diagnosis is common in our case series, which might be involved in the increasing numbers of IEM among children that in many cases death even before getting results.

Since we need to probably way forward for our country, Therefore, the Standardisation of AA by TLC was applied in sera patients of suspected IEM. If the screening procedure is properly applied, the tentative diagnosis of Amino acids disorder might be possible in the same day of the test.

Patient's samples were selected from Kerbala teaching hospital for children. Study consists of 56 patients suspected with inborn errors of metabolism, which were selected from the rare diseases unit.

Samples were analyse for any amino acid disorder using TLC exactly same as method of the standard of AA, and in the same time, in order to confirm the results of TLC, a blood samples of some patients (12 cases) were sent for screening using Tandem Mass Spectrometry (TMS) to Amman/ Jorden. The results were back after 1 month. Samples of TLC run for separation of AA in urine and serum of suspected patients with IEM shown in figure (3-16),(3-17) (3-18)(3-19) and TMS for same patient were illustrated in the appendix shown in figures(21,22,23). Neither the blood samples nor the urine samples of the suspected patients were demonstrated any AA disorder compering to the colour and shapes of the standard AA, these patients' results for no AA disorder were confirmed by patient's results of mass spectrum.

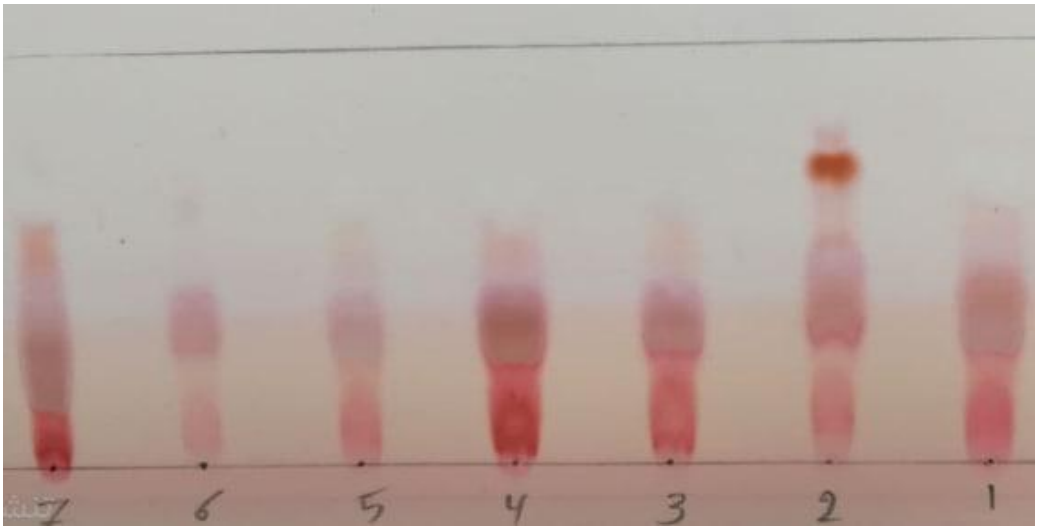


Figure 3-16 TLC run using urine samples of suspected patients with IEM

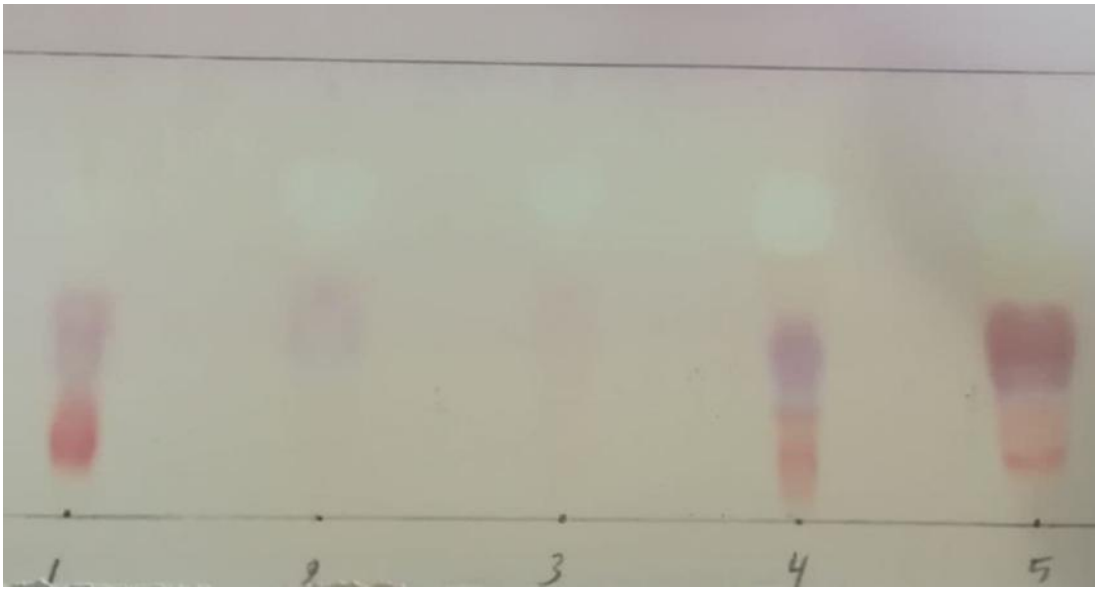


Figure 3-17 TLC run using urine samples of suspected patients with IEM

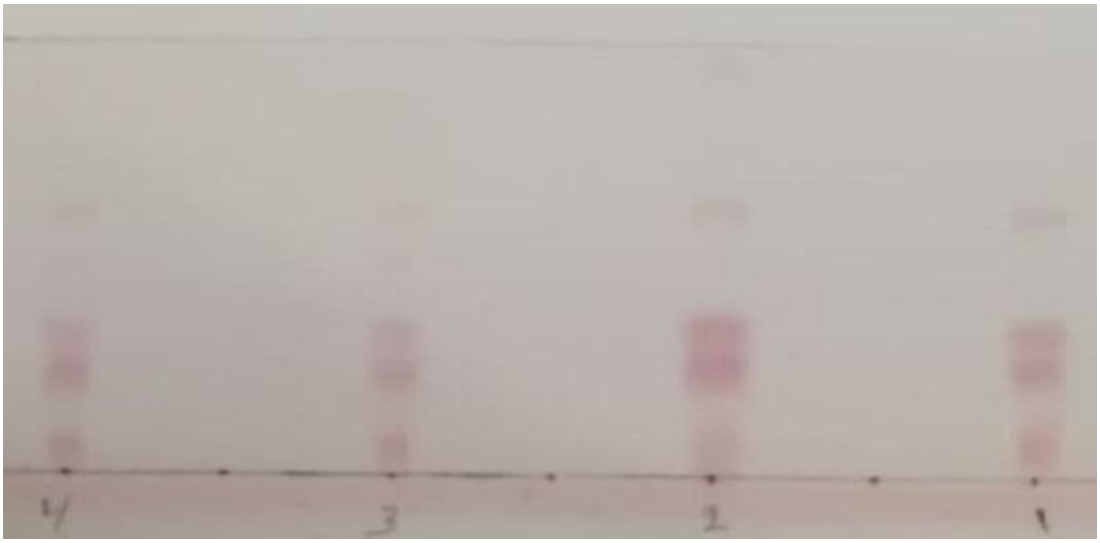


Figure 3-18 TLC run using serum samples of suspected patients with IEM

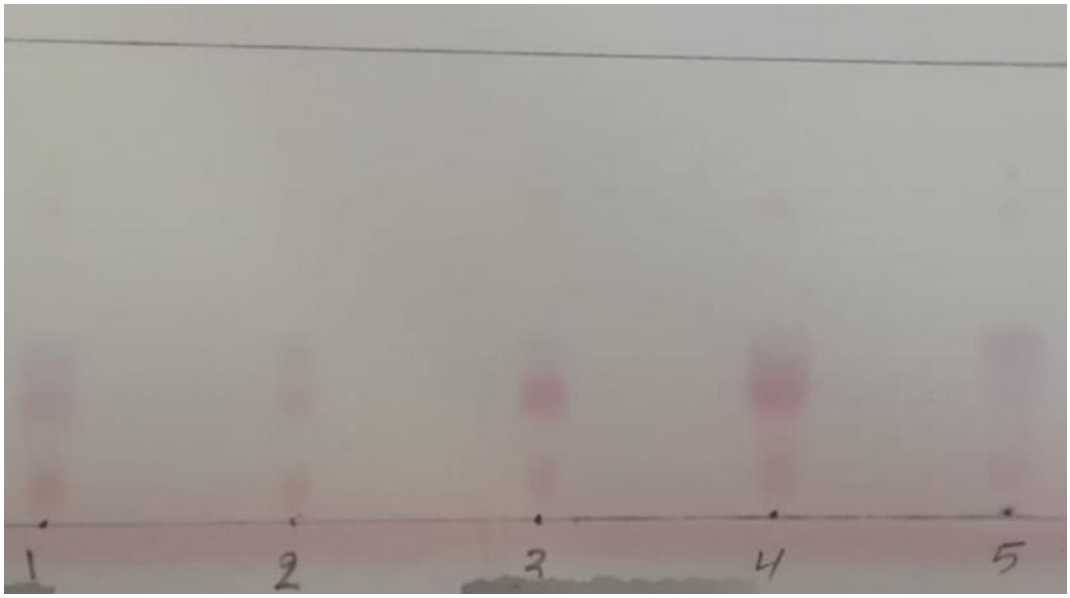


Figure 3-19 TLC run using serum samples of suspected patients with IEM

Chapter Four

Conclusion

And

Future Work

4. Conclusion and Recommendation

4.1. Final conclusion:

The initial tests to perform in a patient suspected to have a type of IEM generally consist of simple tests those could be done in majority of the medical centres, Advanced Investigations are generally performed in specialized centres for IEM and help to carry differential diagnosis further,

NBS is a very important act for the earliest diagnosis of patients with IEM and is of significant importance to initiate early treatment to prevent morbidity and mortality.

The development of analytical methods to measure amino acid concentrations in biological samples can contribute to research on the physiological actions of amino acids and the prediction.

Thin layer chromatography (TLC) was an easy qualitative method, convenient and inexpensive way that could use to determine types of components (AA) were in a mixture of suspected IEM patients by standarazation twenty amino acid.

4.2. Recommendation

Continuous research is needed to identify additional work such as:

1. Much of the pathology in disorders of IEM is known to be caused by oxidative damage by reactive oxygen species (ROS). It could be a good idea to link the type of amino acid disorders with the levels of reactive oxygen species.

2. Confirmed AA disorder samples could be link with genetic and molecular analysis to give more specific investigations.

References

5. References:

1. AGANA, Marisha, Frueh, J., Kamboj, M.,et al. Common metabolic disorder (inborn errors of metabolism) concerns in primary care practice. Annals of translational medicine, 2018, 6.24.

2. J ESPEJO, Angela, F Malaver, L., Rodriguez, A., et al. Recent Patents in Diagnosis and Treatment for Inborn Errors of Metabolism. Recent Patents on Endocrine, Metabolic & Immune Drug Discovery, 2010, 4.2: 111-130.

3. ROMÃO, Andressa, Simon, P. E. A., Góes, J. E. C.,et al. Initial Clinical Presentation in Cases of Inborn Errors of Metabolism in a Reference Children's Hospital: Still a Diagnostic Challenge. Revista Paulista de Pediatria, 2017, 35.3: 258.

4. WERTHEIM-TYSAROWSKA, Katarzyna, Gos, M., Sykut-Cegielska, J., et al. Genetic analysis in inherited metabolic disorders--from diagnosis to treatment. Own experience, current state of knowledge and perspectives. Dev. Period. Med, 2015, 19: 413-431.

5. TIWARI, Shivani; KALLIANPUR, Divya; DESILVA, Kelly Ann. Communication impairments in children with inborn errors of metabolism: A preliminary study. Indian journal of psychological medicine, 2017, 39.2: 146-151.

6. SHAKYA, Arvind Kumar. Advances in Biochemistry & Applications in Medicine, 2017, 3:2-33.

7. DAS, Subir Kumar. Inborn errors of metabolism: Challenges and management,2013,: 311-313.

8. MAK, Chloe Miu, Lee, H. C. H., Chan, A. Y. W.,et al. Inborn errors of metabolism and expanded newborn screening: review and update. Critical reviews in clinical laboratory sciences, 2013, 50.6: 142-162.

9. TURKEL, Susan Beckwitt; WONG, Derek; RANDOLPH, Linda. Psychiatric Symptoms Associated with Inborn Errors of Metabolism. SN Comprehensive Clinical Medicine, 2020, 1-15.

10. ALTIMIMI, Hassan A.; ALJAWADI, Hussein F.; ALI, Esraa A. Inborn Errors of Metabolism in Children with Unexplained Developmental Delay in Misan, Iraq. Oman medical journal, 2019, 34.4: 297.

11. ARIF, Hala S.; THEJEAL, Rabab F.; FARHAN, Ahmed. Inborn errors of metabolism status in Iraq. IOSR J Pharm Biol Sci, 2016, 11: 58-62.

12. ABBAS, Ashwaq N.; HASAN, Mwafak K.; AHMED, Muhammad A. Frequency inborn error of mitochondrial function in Mosul and Kurdistan region. Iraqi Journal of Pharmaceutical Sciences (P-ISSN: 1683-3597, E-ISSN: 2521-3512), 2020, 29.2: 259-262.

13. ALI, WH Al Bu, et al. Risk factors and birth prevalence of birth defects and inborn errors of metabolism in Al Ahsa, Saudi Arabia. Pan African Medical Journal, 2011, 8.1.

14. AL RIYAMI, Sulaiman, et al. Detection of inborn errors of metabolism using tandem mass spectrometry among high-risk Omani patients. Oman medical journal, 2012, 27.6: 482.

15. GOLBAHAR, J., Al-Jishi, E. A., Altayab, D. D., et al. Selective newborn screening of inborn errors of amino acids, organic acids and fatty acids metabolism in the Kingdom of Bahrain. Molecular genetics and metabolism, 2013, 110.1-2: 98-101.

16. SELIM, Laila A. Hassan, S. A. H., Salem, F.,, et al. Selective screening for inborn errors of metabolism by tandem mass spectrometry in Egyptian children: a 5 year report. Clinical biochemistry, 2014, 47.9: 823-828.

17. ALFADHEL, Majid; BABIKER, Amir. Inborn errors of metabolism associated with hyperglycaemic ketoacidosis and diabetes mellitus: narrative review. Sudanese journal of paediatrics, 2018, 18.1: 10.

18. KHALAF, Shaimaa Mohamed, El-Tellawy, M. M., Refat, N. H., et al. Detection of some metabolic disorders in suspected neonates admitted at Assiut University Children Hospital. Egyptian Journal of Medical Human Genetics, 2019, 20.1: 1-7.

19. KWON, Jennifer M. Testing for inborn errors of metabolism. Continuum: Lifelong Learning in Neurology, 2018, 24.1: 37-56.

20. VILLANI, Guglielmo RD, Gallo, G., Scolamiero, E.,et al. "Classical organic acidurias": diagnosis and pathogenesis. Clinical and experimental medicine, 2017, 17.3: 305-323.

21. MATSUMOTO, Shirou, Häberle, J., Kido, J., et al. Urea cycle disorders—update. Journal of human genetics, 2019, 64.9: 833-847.

22. BLAIR, Nicholas F.; CREMER, Philip D.; TCHAN, Michel C. Urea cycle disorders: a life-threatening yet treatable cause of metabolic encephalopathy in adults. Practical neurology, 2015, 15.1: 45-48.

23. BLAU, Nenad, Duran, M., Gibson, K. M., et al. (ed.). Physician's guide to the diagnosis, treatment, and follow-up of inherited metabolic diseases. Heidelberg:: Springer, 2014.

24. VISHWANATH, Vijay A. Fatty acid beta-oxidation disorders: a brief review. Annals of neurosciences, 2016, 23.1: 51-55.

25. BOTTANI, Emanuela, Lamperti, C., Prigione, A., et al. Therapeutic Approaches to Treat Mitochondrial Diseases:"One-Size-Fits-All" and "Precision Medicine" Strategies. Pharmaceutics, 2020, 12.11: 1083.

26. SOLOMON, Melani; MURO, Silvia. Lysosomal enzyme replacement therapies: historical development, clinical outcomes, and future perspectives. Advanced drug delivery reviews, 2017, 118: 109-134.

27. FERDINANDUSSE, Sacha, Jimenez-Sanchez, G., Koster, J., et al. A novel bile acid biosynthesis defect due to a deficiency of peroxisomal ABCD3. Human molecular genetics, 2015, 24.2: 361-370.

28. MONOSTORI, Péter, Klinke, G., Hauke, J., et al. Extended diagnosis of purine and pyrimidine disorders from urine: LC MS/MS assay development and clinical validation. PloS one, 2019, 14.2: e0212458.

29. BIERAU, Jörgen; ŠEBESTA, Ivan. Purine and pyrimidine disorders. In: Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases. Springer, Berlin, Heidelberg, 2014. p. 641-660.

30. EDEL, Yonatan; MAMET, Rivka. Porphyria: what is it and who should be evaluated?. Rambam Maimonides medical journal, 2018, 9.2.

31. VAN DEN AKKERD, Chris H.; DE GROOFE, Femke; VAN DER SCHOORC, Sophie RD. Amino Acids and Proteins. Nutritional Care of Preterm Infants: Scientific Basis and Practical Guidelines, 2014, 110: 49-63.

32. ALIU, Ermal; KANUNGO, Shibani; ARNOLD, Georgianne L. Amino acid disorders. Annals of translational medicine, 2018, 6.24.

33. HASSANIEN, Mohamed Fawzy Ramadan. Food Proteins and Enzymes ,2012,:1-55.

34. RAZAK, Meerza Abdul, Begum, P. S., Viswanath, B., et al. Multifarious beneficial effect of nonessential amino acid, glycine: a review. Oxidative medicine and cellular longevity, 2017, 2017.

35. BUBBER, Parvesh. Unit-1 Amino Acids, Peptides and Proteins. Indira Gandhi National Open University, New Delhi,2021,:7-28.

36. METCALF, J. S., Dunlop, R. A., Powell, J. T., et al. L-serine: a naturally-occurring amino acid with therapeutic potential. Neurotoxicity research, 2018, 33.1: 213-221.

37. OLOGHOBO-UI, Anthony IO; JOKTHAN–NOUN, Grace E.; NJIDDA–NOUN, Ahmed A. Course Team.

38. PETERSEN, Kitt Falk, Dufour, S., Cline, G. W., et al. Regulation of hepatic mitochondrial oxidation by glucose-alanine cycling during starvation in humans. The Journal of clinical investigation, 2019, 129.11: 4671-4675.

39. GHOSH, Prahlad C. Amino acid metabolism,2008.:1-46.

40. SHEN, Fan; SERGI, Consolato. Biochemistry, Amino Acid Synthesis and Degradation. StatPearls [Internet], 2021.

41. PAUL, Bindu D.; SBODIO, Juan I.; SNYDER, Solomon H. Cysteine metabolism in neuronal redox homeostasis. Trends in pharmacological sciences, 2018, 39.5: 513-524.

42. WANG, Yifeng, et al. Bile acids regulate cysteine catabolism and glutathione regeneration to modulate hepatic sensitivity to oxidative injury. JCI insight, 2018, 3.8.

43. QIU, Xue-Mei, et al. Signaling role of glutamate in plants. Frontiers in plant science, 2020, 10: 1743.

44. SCHOUSBOE, Arne; BAK, Lasse Kristoffer; WAAGEPETERSEN, Helle Sønderby. Astrocytic control of biosynthesis and turnover of the neurotransmitters glutamate and GABA. Frontiers in endocrinology, 2013, 4: 102.

45. CANFIELD, Clare-Ann; BRADSHAW, Patrick C. Amino acids in the regulation of aging and aging-related diseases. Translational Medicine of Aging, 2019, 3: 70-89.

46. OLOGHOBO-UI, Anthony IO; JOKTHAN–NOUN, Grace E.; NJIDDA–NOUN, Ahmed A. Course Team.

47. YAHYAOUI, Raquel; PÉREZ-FRÍAS, Javier. Amino acid transport defects in human inherited metabolic disorders. International journal of molecular sciences, 2020, 21.1: 119.

48. LEANDRO, João; HOUTEN, Sander M. The lysine degradation pathway: Subcellular compartmentalization and enzyme deficiencies. Molecular GeneticsandMetabolism,2020, (https://doi.org/10.1016/j.ymgme.2020.07.010).

49. OGNIK, Katarzyna, Całyniuk, Z., Mikulski, D.,et al. The effect of different dietary ratios of lysine, arginine and methionine on biochemical parameters and hormone secretion in turkeys. Journal of Animal Physiology and Animal Nutrition, 2021, 105.1: 108-118.

50. JUNG, Yanghoon P. Comprehensive Assessment of a Pre-Workout Dietary Supplement with and without Synephrine. 2016. PhD Thesis.

51. BAYNES, John W. Aging of complex systems. Medical Biochemistry E-Book, 2018, 416.

52. SPRUCE, Neal F.; TITCHENAL, C. Alan. 15 Other Individual Amino. Sports Nutrition: Fats and Proteins, 2007, 279.

53. DEMIRBAS, Didem; BRUCKER, William J.; BERRY, Gerard T. Inborn errors of metabolism with hepatopathy: metabolism defects of galactose, fructose, and tyrosine. Pediatric Clinics, 2018, 65.2: 337-352.

54. BADAWY, Abdulla AB. Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects. International Journal of Tryptophan Research, 2017, 10: 1178646917691938.

55. KESSLER, Aleeza T.; RAJA, Avais. Biochemistry, histidine. StatPearls [Internet], 2019.

56. CAPPELLETTI, Pamela, Tallarita, E., Rabattoni, V., et al. Proline oxidase controls proline, glutamate, and glutamine cellular concentrations in a U87 glioblastoma cell line. PloS one, 2018, 13.4: e0196283.

57. ADEVA-ANDANY, María M., López-Maside, L., Donapetry-García, C., et al. Enzymes involved in branched-chain amino acid metabolism in humans. Amino acids, 2017, 49.6: 1005-1028.

58. NIE, Cunxi, et al. Branched chain amino acids: beyond nutrition metabolism. International journal of molecular sciences, 2018, 19.4: 954.

59. MacDonald, A.Phenylketonuria. Reference Module in Life Sciences,2017, doi:10.1016/B978-0-12-809633-8.06896-5.

60. DIXON, Marjorie; MACDONALD, Anita; WHITE, Fiona J. Disorders of amino acid metabolism, organic acidaemias and urea cycle disorders. Clinical Paediatric Dietetics, 2020, 513-598.

61. ALSHARHAN, Hind; FICICIOGLU, Can. Disorders of phenylalanine and tyrosine metabolism. Translational Science of Rare Diseases, Preprint: 1-56.

62. RANGANATH, Lakshminarayan R.; NORMAN, Brendan P.; GALLAGHER, James A. Ochronotic pigmentation is caused by homogentisic acid and is the key event in alkaptonuria leading to the destructive consequences of the disease—a review. Journal of inherited metabolic disease, 2019, 42.5: 776-792.

63. PATEL, Khushbu; MASTER, Stephen R. Newborn screening and inborn errors of metabolism. In: Contemporary Practice in Clinical Chemistry. Academic Press, 2020. p. 861-879.

64. HUONG, Nguyen Thi Thu, Dung, V. C., Ngan, N. T. T., et al. Hereditary characteristics of the S339L mutation in a patient with maple syrup urine disease in Vietnam. Academia Journal of Biology, 2020, 42.2.

65. KAUR, Rajdeep, Attri, S. V., Saini, A. G., et al. Seven novel genetic variants in a North Indian cohort with classical homocystinuria. Scientific reports, 2020, 10.1: 1-9.

66. DEARMOND, P. D.; DIETZEN, D. J.; PYLE-EILOLA, A. L. Amino acids disorders. Biomarkers in inborn errors of metabolism: clinical aspects and laboratory determination. 1st ed. Elsevier, Inc, 2017, 25-64.

67. KOENS, Lisette H., Tijssen, M. A., Lange, F., et al. Eye movement disorders and neurological symptoms in late- onset inborn errors of metabolism. Movement Disorders, 2018, 33.12: 1844-1856.

68. FERREIRA, Carlos R.; VAN KARNEBEEK, Clara DM. Inborn errors of metabolism. Handbook of clinical neurology, 2019, 162: 449-481.

69. VASSILI, Valayannopoulos; TIEN, Poll-The Bwee. Diagnostic work-up in acute conditions of inborn errors of metabolism and storage diseases. Handbook of clinical neurology, 2013, 113: 1553-1562.

70. VASSILI, Valayannopoulos; TIEN, Poll-The Bwee. Diagnostic work-up in acute conditions of inborn errors of metabolism and storage diseases. Handbook of clinical neurology, 2013, 113: 1553-1562.

71. SIRRS, S. M., Lehman, A., Stockler, S., et al. Treatable inborn errors of metabolism causing neurological symptoms in adults. Molecular genetics and metabolism, 2013, 110.4: 431-438.

72. Ruben Bonilla Guerrero, Denise Salaza., Pranoot Tanpaiboon .diagnostic approaches in metabolic disorders. Annals of translational medicine, 2018, 6.24. doi: 10.21037/atm.2018.11.05

73. TROISI, Jacopo, Cavallo, P., Colucci, A., et al. Metabolomics in genetic testing. Advances in clinical chemistry, 2020, 94: 85-153.

74. VIOLI, Jake P., Bishop, D. P., Padula, M. P., et al. Considerations for amino acid analysis by liquid chromatography-tandem mass spectrometry: A tutorial review. TrAC Trends in Analytical Chemistry, 2020, 116018.

75. RENITAA, A. Annam, Kumarb, P. S., Srinivasb, S., et al. A review on analytical methods and treatment techniques of pharmaceutical wastewater. Desalination and Water Treatment, 2017, 87: 160-178.

76. KUMAR, Sanjeet; JYOTIRMAYEE, K.; SARANGI, Monalisa. Thin layer chromatography: a tool of biotechnology for isolation of bioactive

compounds from medicinal plants. International Journal of Pharmaceutical Sciences Review and Research, 2013, 18.1: 126-132.

77. GUEVARA, Govinda; PERERA, Julián; NAVARRO-LLORENS, Juana-María. Analysis of Intermediates of Steroid Transformations in Resting Cells by Thin-Layer Chromatography (TLC). In: Microbial Steroids. Humana Press, New York, NY, 2017. p. 347-360.

78. KALÁSZ, Huba; BÁTHORI, Mária; VALKÓ, Klára L. Basis and pharmaceutical applications of thin-layer chromatography. In: Handbook of Analytical Separations. Elsevier Science BV, 2020. p. 523-585.

79. AMOLI-DIVA, Mitra; POURGHAZI, Kamyar. Gold nanoparticles grafted modified silica gel as a new stationary phase for separation and determination of steroid hormones by thin layer chromatography. Journal of food and drug analysis, 2015, 23.2: 279-286.

80. WILSON, Keith; WALKER, John (ed.). Principles and techniques of biochemistry and molecular biology. Cambridge university press, 2010.

81. SHEETAL, Gadekar; ARCHANA, Tiwari. QUANTITATIVE ESTIMATION OF CEFOTAXIME SODIUM BY RP–HP IN BULK AND PHARMACEUTICAL DOSAGE FORM. 2020.

82. BHOJ, Yogita, Tharmavaram, M., Pandey, G., et al. Chromatographic Techniques for Forensic Investigations. Technology in Forensic Science: Sampling, Analysis, Data and Regulations, 2020, 129-149.

83. BÜYÜKKÖROĞLU, Gülay, Dora, D. D., Özdemir, F., et al. Techniques for protein analysis. In: Omics Technologies and Bio-Engineering. Academic Press, 2018. p. 317-351.

84. CAMARASA, Cristina Gómez; COBO, Fernando. Application of MALDI-TOF mass spectrometry in clinical virology. In: The Use of Mass Spectrometry Technology (MALDI-TOF) in Clinical Microbiology. Academic Press, 2018. p. 167-180.

85. The data analysis for this paper was generated using the Real Statistics Resource Pack software (Release 7.6). Copyright (2013 – 2021) Charles Zaiontz. www.real-statistics.com.

86. AFZAL, Raja Majid; LUND, Allan Meldgaard; SKOVBY, Flemming. The impact of consanguinity on the frequency of inborn errors of metabolism. Molecular genetics and metabolism reports, 2018, 15: 6-10.

87. ALTIMIMI, Hassan A.; ALJAWADI, Hussein F.; ALI, Esraa A. Inborn Errors of Metabolism in Children with Unexplained Developmental Delay in Misan, Iraq. Oman medical journal, 2019, 34.4: 297.

88. FITZGERALD, Marianne; CRUSHELL, Ellen; HICKEY, Caroline. Cyclic vomiting syndrome masking a fatal metabolic disease. European journal of pediatrics, 2013, 172.5: 707-710.

89. SCHILLACI, Lori-Anne P.; DEBROSSE, Suzanne D.; MCCANDLESS, Shawn E. Inborn errors of metabolism with acidosis: Organic acidemias and defects of pyruvate and ketone body metabolism. Pediatric Clinics, 2018, 65.2: 209-230.

90. YILDIZ, Yılmaz; SIVRI, Hatice Serap. Inborn errors of metabolism in the differential diagnosis of fatty liver disease. The Turkish Journal of Gastroenterology, 2020, 31.1: 3.

91. SALEEM, Tahia H.; HASSAN, Mohammed H. Map of some inborn errors of metabolism in Upper Egypt: Metabolic and Genetic Disorders' unit, ten

years' experience. East African Scholars Journal of Medical Sciences, 2019, 2.6: 306-10.

92. SAUDUBRAY, Jean-Marie; GARCIA-CAZORLA, Àngels. Inborn Errors of Metabolism Overview: Pathophysiology, Manifestations, Evaluation, and Management. Pediatric Clinics of North America, 2018, 65.2: 179-208.

93. SAUDUBRAY, Jean-Marie; GARCIA-CAZORLA, Angels. Clinical approach to inborn errors of metabolism in pediatrics. In: Inborn metabolic diseases. Springer, Berlin, Heidelberg, 2016. p. 3-70.

94. EL-GHARBAWY, Areeg; VOCKLEY, Jerry. Inborn errors of metabolism with myopathy: defects of fatty acid oxidation and the carnitine shuttle system. Pediatric Clinics, 2018, 65.2: 317-335.

95. KÖLKER, Stefan, Cazorla, A. G., Valayannopoulos, V., Lund, et al. The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: the initial presentation. Journal of Inherited Metabolic Disease: Official Journal of the Society for the Study of Inborn Errors of Metabolism, 2015, 38.6: 1041-1057.

96. EL-HATTAB, Ayman W. Inborn errors of metabolism. Clinics in perinatology, 2015, 42.2: 413-439.DOI: 10.1016/j.clp.2015.02.010.

97. SHARMA, Suvasini; PRASAD, Asuri N. Inborn errors of metabolism and epilepsy: current understanding, diagnosis, and treatment approaches. International journal of molecular sciences, 2017, 18.7: 1384.

98.ASADI-POOYA, Ali A. Semiological classification of psychogenic nonepileptic seizures: a systematic review and a new proposal. Epilepsy & Behavior, 2019, 100: 106412.

99. SALEEM, Tahia H., Rashwan, N. I., Hassan, M. H.,et al. Clinical and Biochemical Characterizations of Pediatric Patients with Urea Cycle Disorders in Upper Egypt: A Case-Control Study. International Journal of Pediatrics, 2020, 8.9: 11945-11957.

100. FÖRDERREUTHER, Stefanie; RUSCHEWEYH, Ruth. From ophthalmoplegic migraine to cranial neuropathy. Current pain and headache reports, 2015, 19.6: 21.

101. LIAO, Kuo-Yu, Chang, F. Y., Wu, L. T., et al. Cyclic vomiting syndrome in Taiwanese children. Journal of the Formosan Medical Association, 2011, 110.1: 14-18.

102. LAI, Kent; BOXER, Matthew B.; MARABOTTI, Anna. GALK inhibitors for classic galactosemia. Future medicinal chemistry, 2014, 6.9: 1003-1015..

103. GITZELMANN, Richard; STEINMANN, Beat. Galactosemia: how does long-term treatment change the outcome. Enzyme, 1984, 32.1: 37-46.

104. SHRIBERG, Lawrence D.; POTTER, Nancy L.; STRAND, Edythe A. Prevalence and phenotype of childhood apraxia of speech in youth with galactosemia. 2011.

105. POTTER, Nancy L.; NIEVERGELT, Yves; SHRIBERG, Lawrence D. Motor and speech disorders in classic galactosemia. In: JIMD Reports-Volume 11. Springer, Berlin, Heidelberg, 2013. p. 31-41.

106. HUGHES, Joanne, et al. Outcomes of siblings with classical galactosemia. The Journal of pediatrics, 2009, 154.5: 721-726.

107. VANGALA, Subrahmanyam; TONELLI, Alfred. Biomarkers, metabonomics, and drug development: can inborn errors of metabolism help in understanding drug toxicity?. The AAPS journal, 2007, 9.3: E284-E297.

108. DE GIORGIS, Valentina; VEGGIOTTI, Pierangelo. GLUT1 deficiency syndrome 2013: current state of the art. Seizure, 2013, 22.10: 803-811.

109. PAPADOPOULOU, Maria, Papadopoulou-Legbelou, K., Koutsampasopoulou, I., et al. Broadening the Picture of Short-Chain Acyl-CoA Dehydrogenase Deficiency: A Case Report with Microcephaly, Leukoencephalopathy, and Characteristic Magnetic Resonance Spectroscopic Findings. Journal of Pediatric Neurology, 2018, 16.04: 243-247.

110. CORNELIUS, Leema P., et al. Pediatric glutaric aciduria type 1: 14 cases, diagnosis and management. Annals of Indian Academy of Neurology, 2021, 24.1: 22.

111. KUMTA, N. B. Inborn errors of metabolism (IEM)—an Indian perspective. The Indian Journal of Pediatrics, 2005, 72.4: 325-332.

Appendix

Appendix

Questionnaire

University of Karbala-college of medicine

Department of clinical biochemistry

Experimental data

Type of study: gross section study

Sample No…….
Age…….
Gender…male…female…….

Body mass index… (Weight and High)…

Do the father and mother relatives?

Do you have other children suffering from symptoms of the same disease?

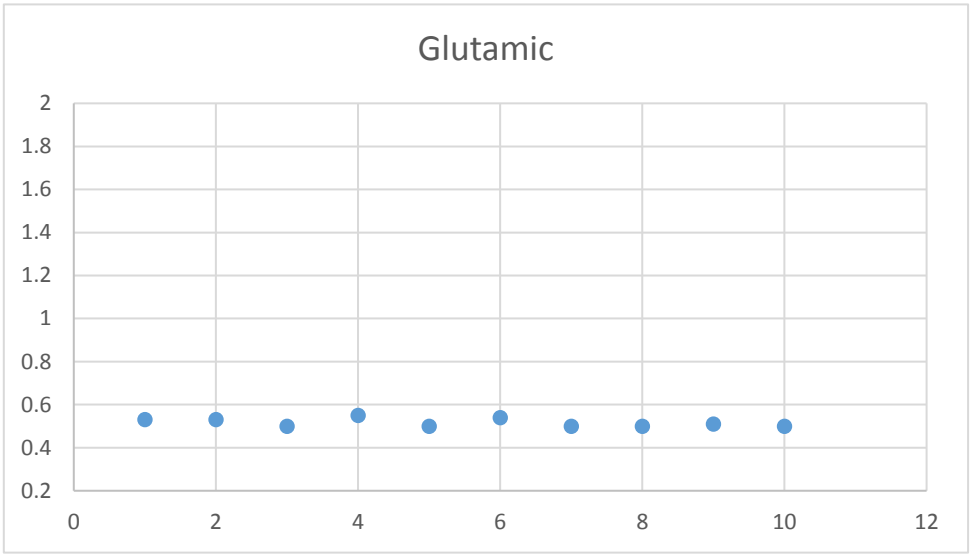
Does the family have any chronic genetic history?

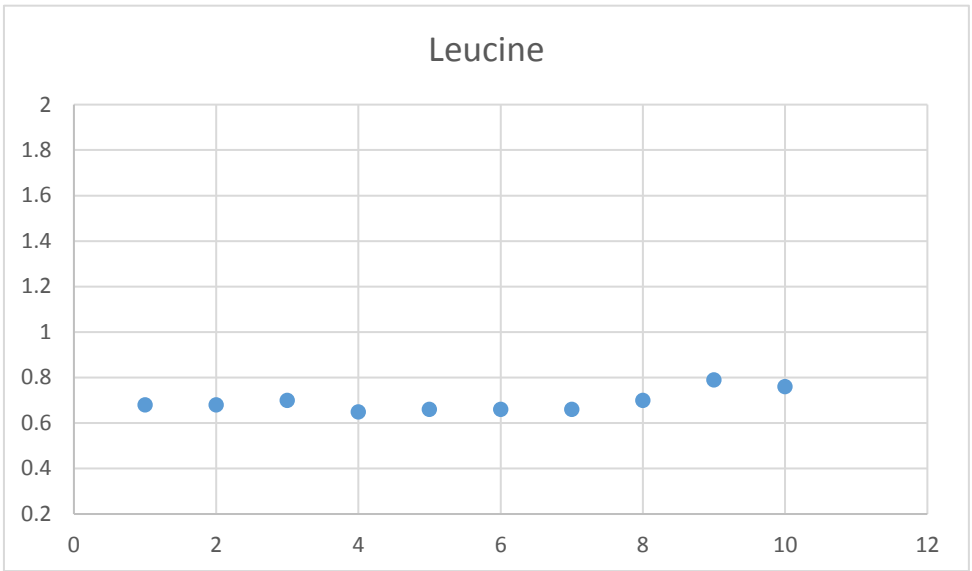
What is the patient's diet?

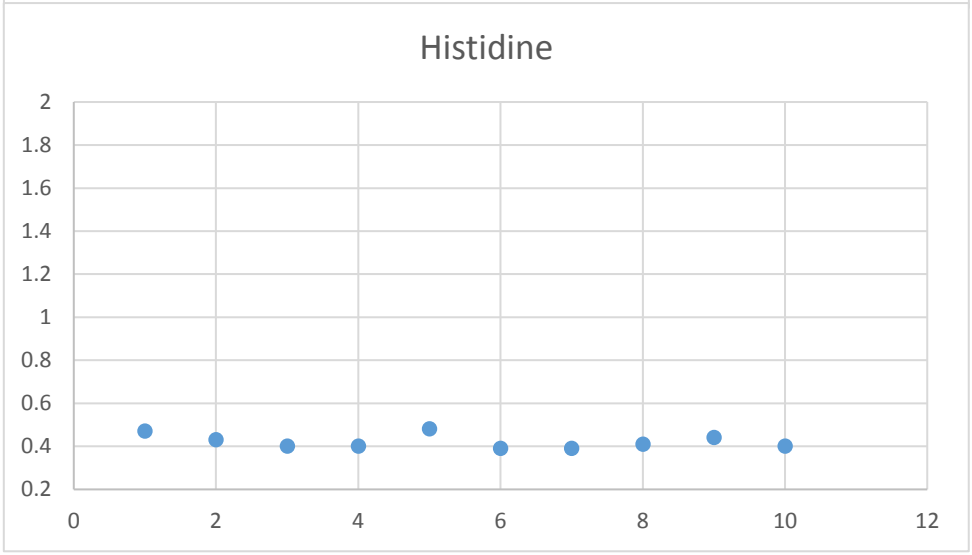
What is the type of feeding Breastfeeding or artificial?

What treatment did mother take during pregnancy?

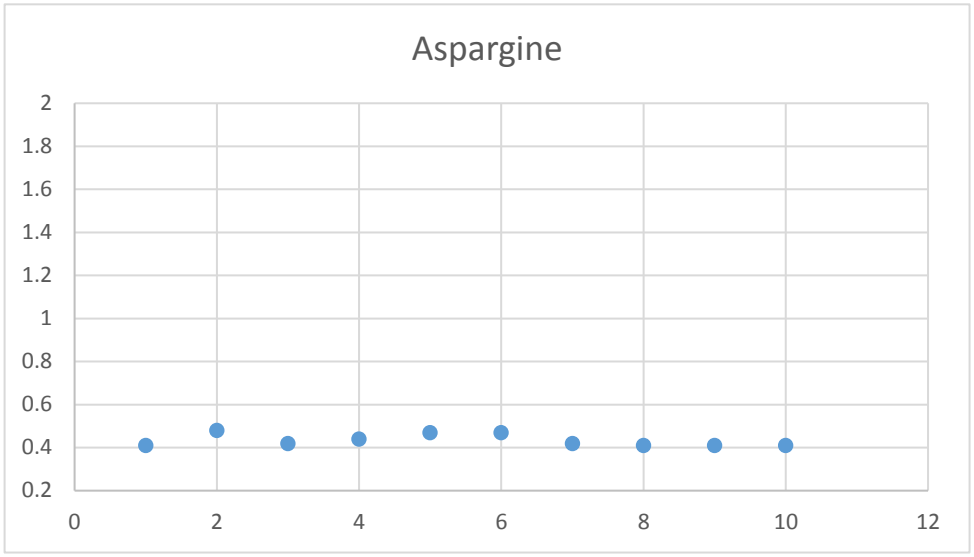
Figures 20: Calculated Rf of standard amino acids from ten repeated runs by TLC.

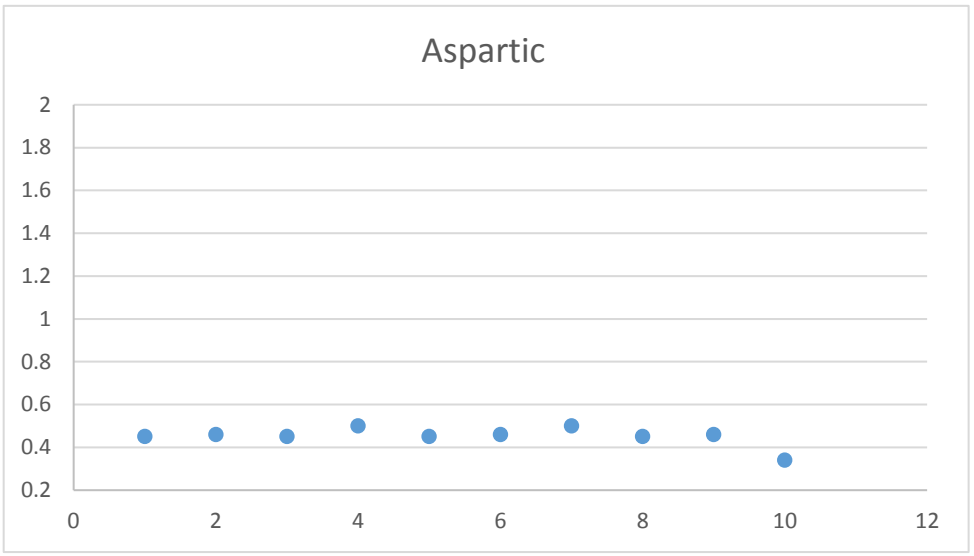


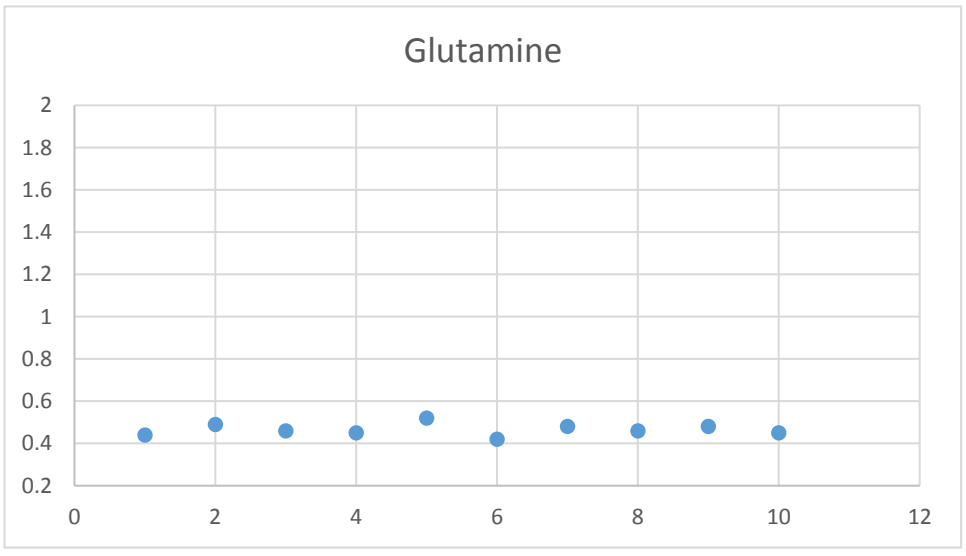




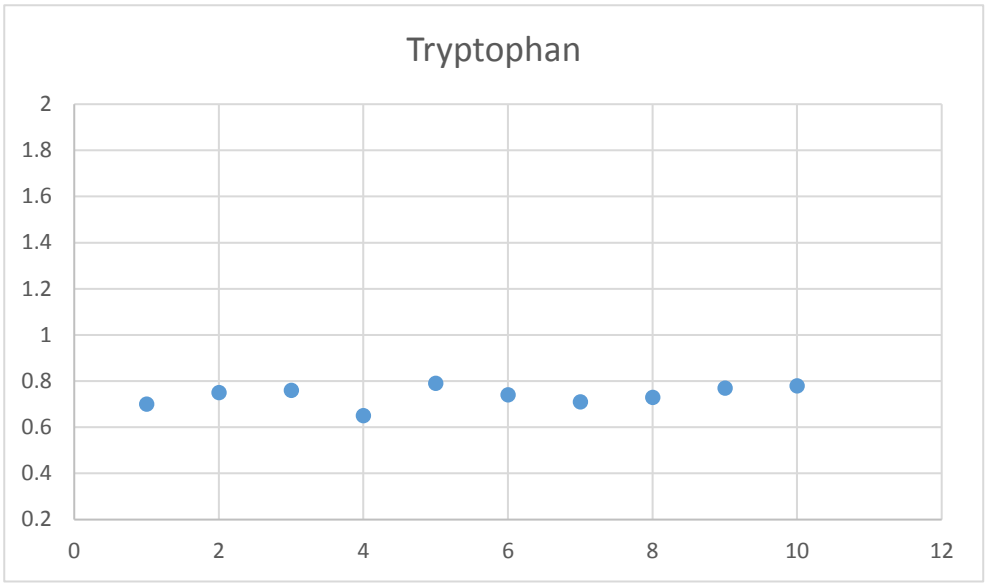
Appendix

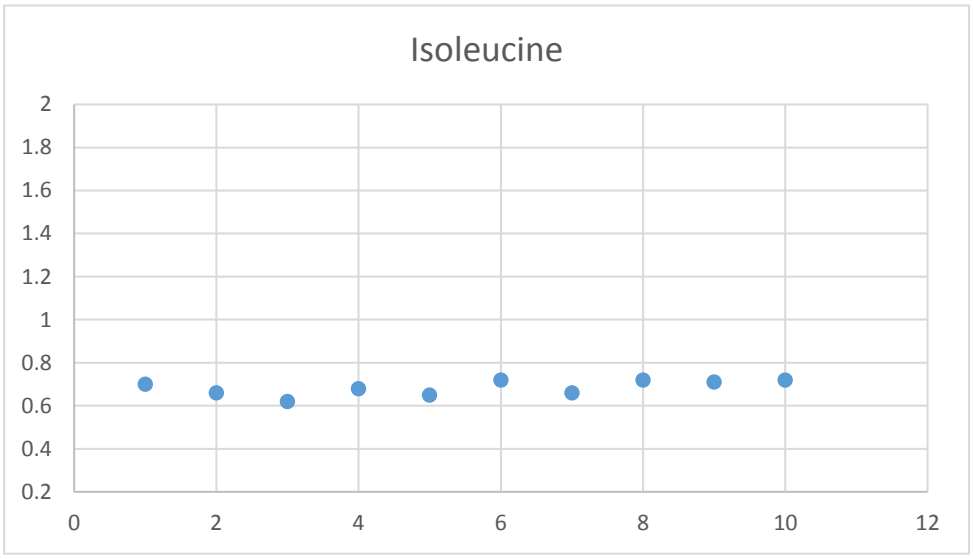

Aspargine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12

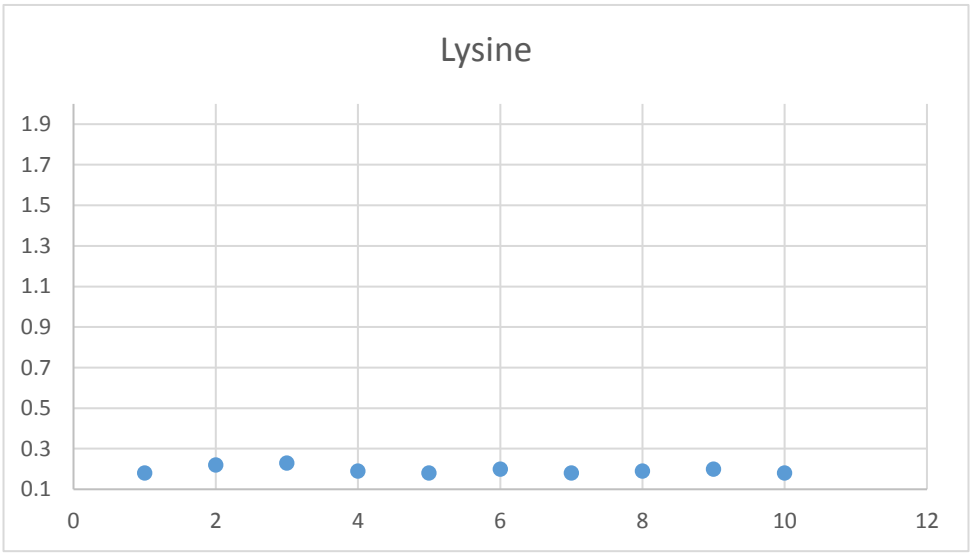

Aspartic
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12


Glutamine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12

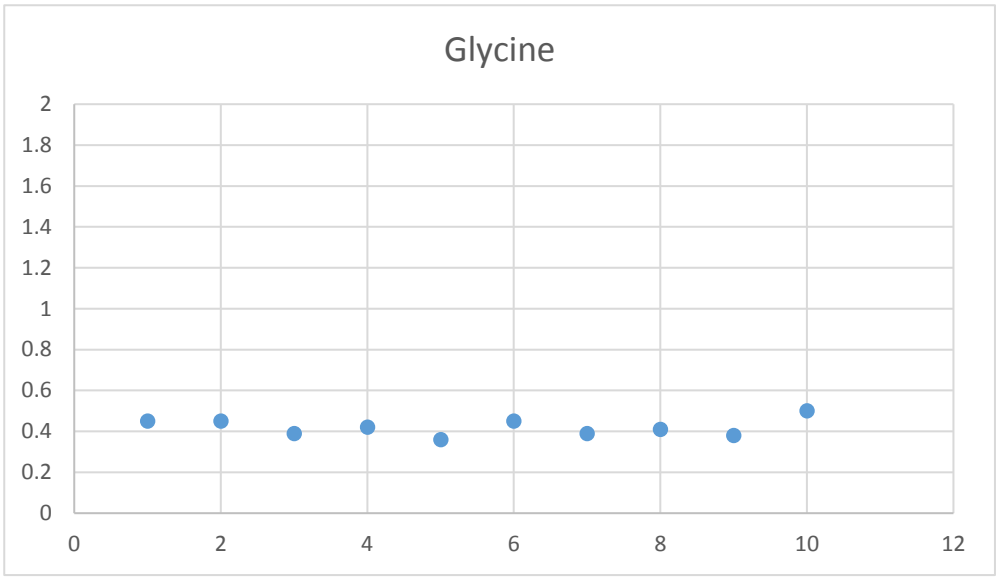
Appendix

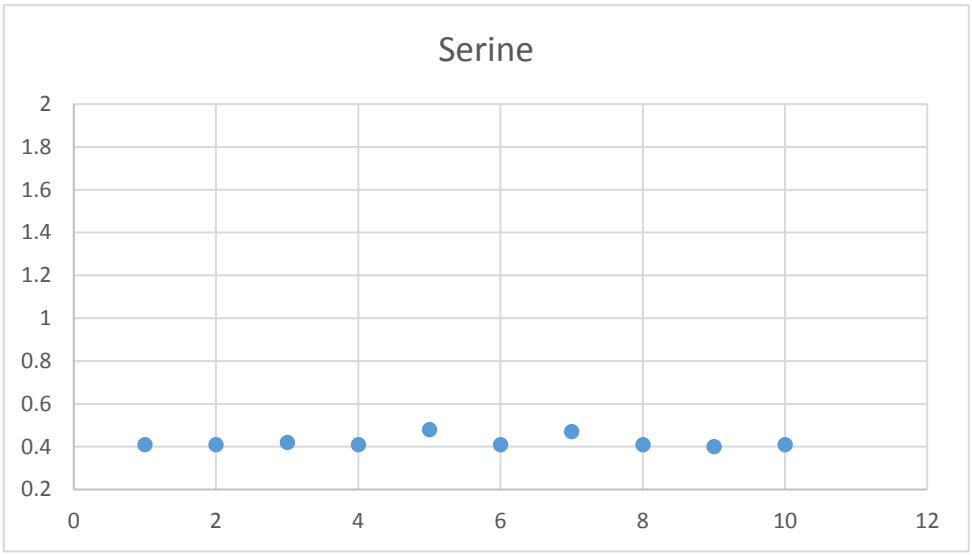

Tryptophan
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12

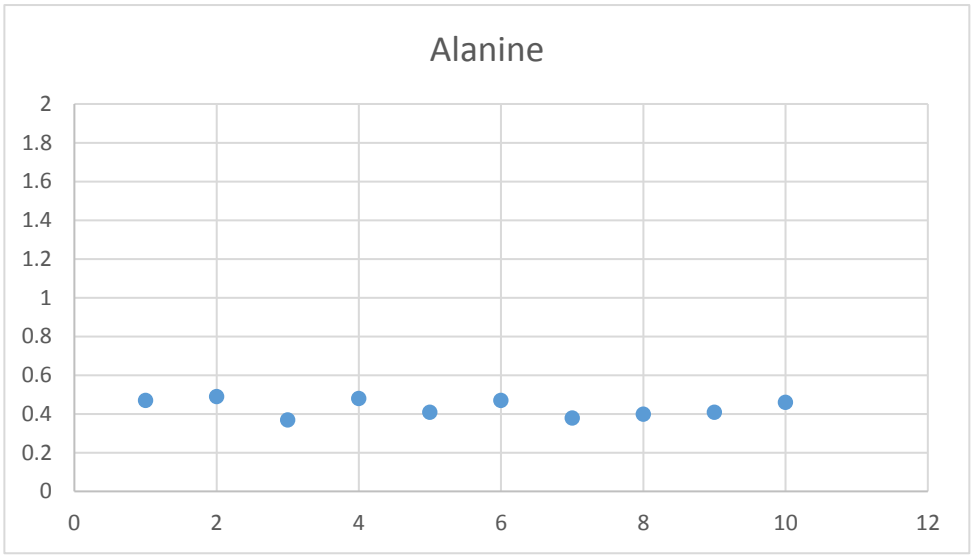

Isoleucine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12


Lysine
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
0
2
4
6
8
10
12

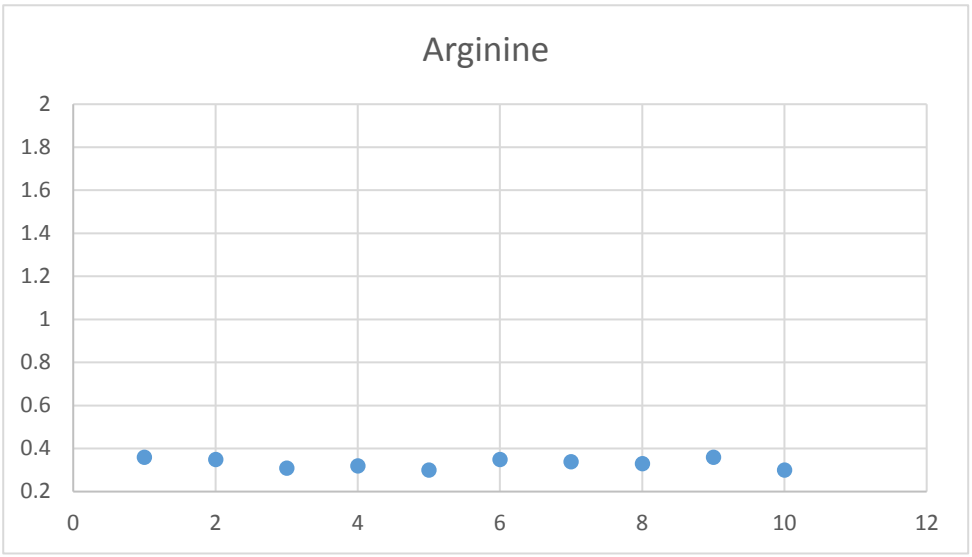
Appendix

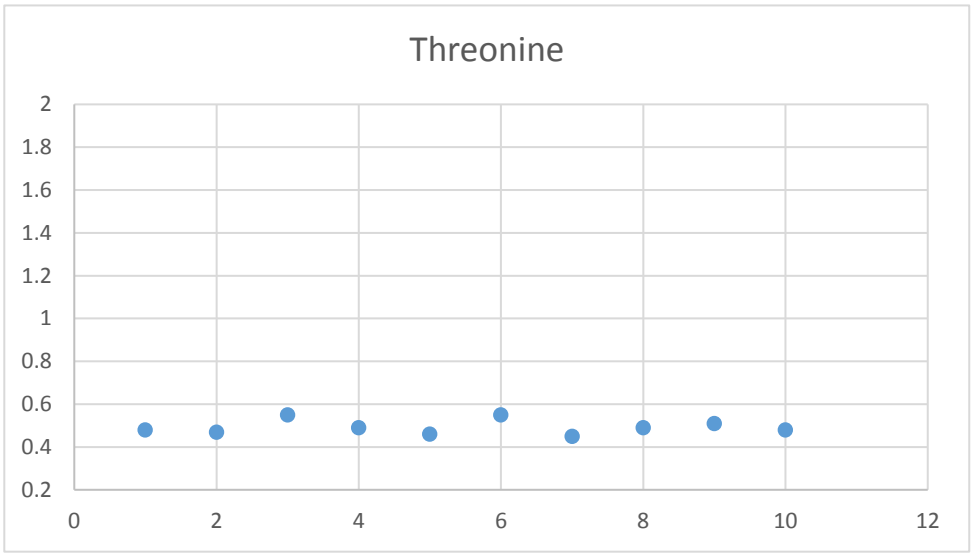

Glycine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12

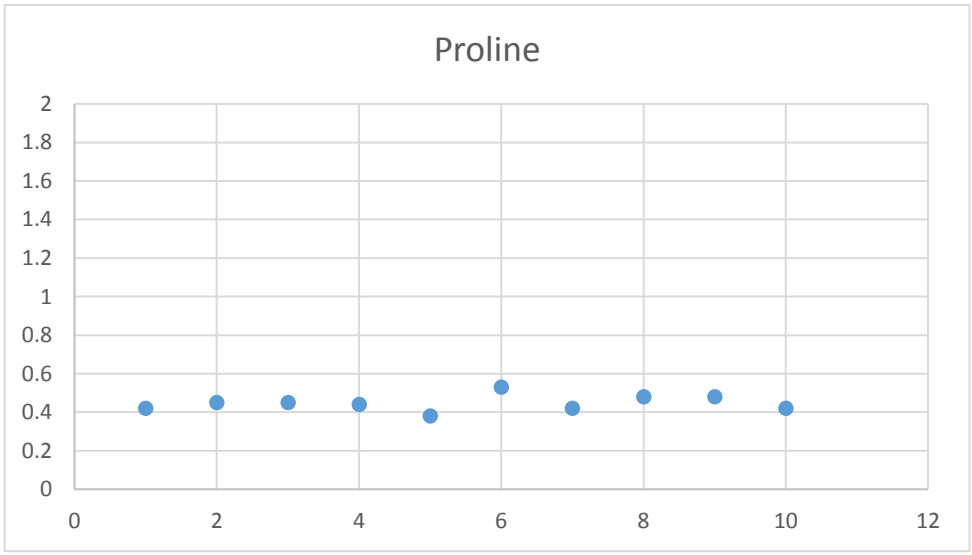

Serine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12


Alanine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12

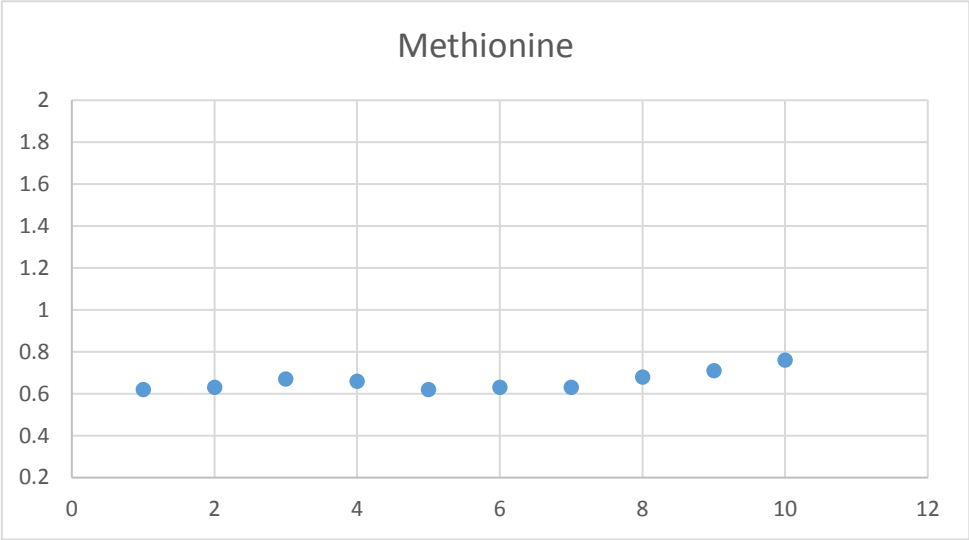
Appendix

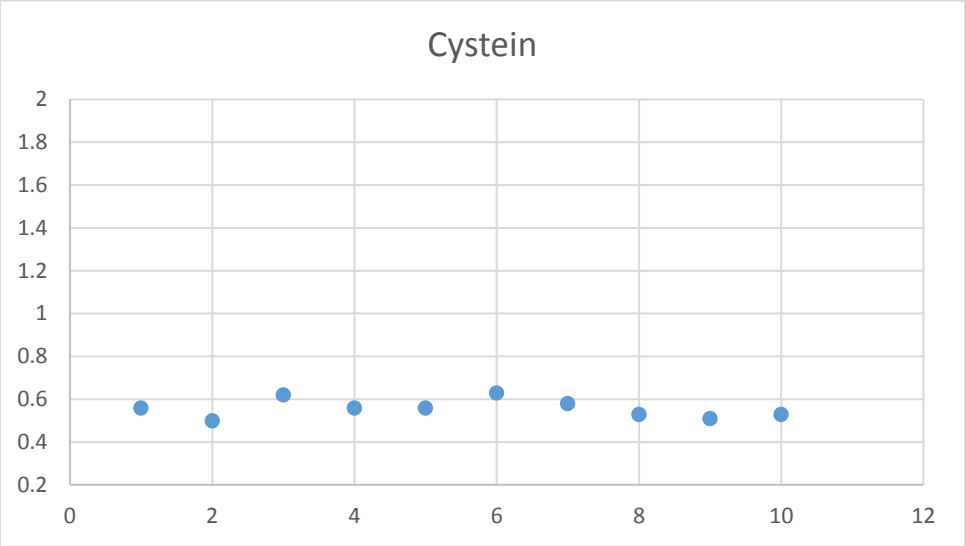

Arginine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12

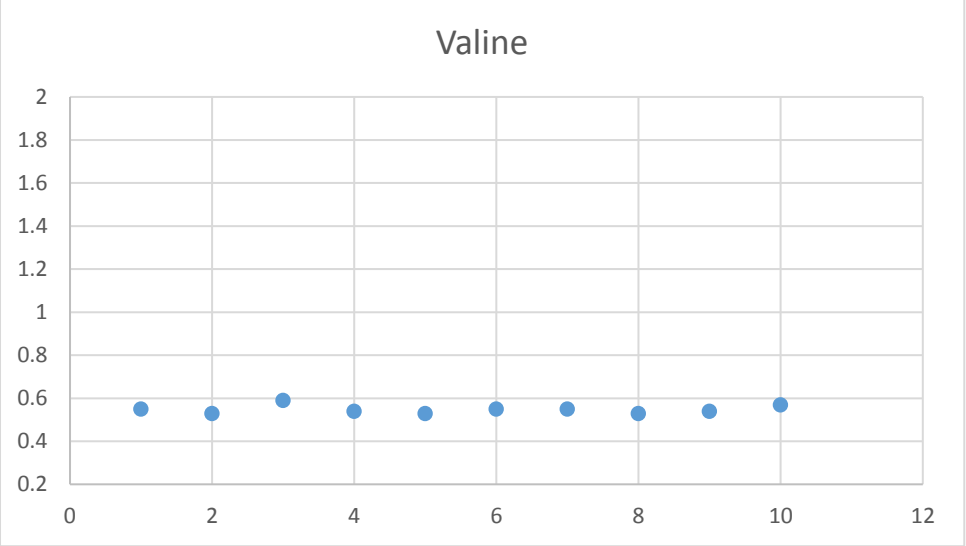

Threonine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12


Proline
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12

Appendix


Methionine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12


Cystein
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12


Valine
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
12

Figures 21 shows the result of patient with TMS

MedLabs Consultancy Group

Clinical Pathology - Referra

CAP

T +962 6 463 0362 | F +962 6 464 031

v. 21- Abu Firas Al-Hamadani St. - 2nd Floor - Jabal Amman - Amman - Jordan | Consultants: Dr. Nashat Dahabreh | Dr. Hassib Sahyoun | Register at the lab to see this result online!

all 0800 22 500 for our house call service | www.medlabsgroup.com | Quality Control: CAP | UKNEQAS | MOH

Baby's Name	: اسيتات فراس جابر				
D.O.B	:06/06/2020	Gender	: Female	Received By	: 20/07/2020
File No	: 20203299110	Visit No	:REF1387665	Sample Date	: 21/06/2020
Consultant	: Noor Al Sabah /Baghda	External No	:17780	Sample Origen	: Iraq
Gestation (wks)	:			Birth Weight (kg)	: 2.500

Newborn Screening Profile 1

Congenital Hypothyroidism (TSH)	: 3.7	mIU/mL	< 9 mIU/mL
Cystic Fibrosis (IRT)	: 69.9	mg/L	< 70 mg/L
Congenital Adrenal Hyperplasia (17-OHP)	: 3.7	nmol/L	< 30 nmol/L
Galactosemia (Total Galactose)	: 1.3	mg/dL	< 7 mg/dL
G6PD Deficiency (G6PD Activity)	: 2.0	U/g Hb	> 2.2 U/g Hb
Hemoglobinopathies	: Normal		

Fatty Acid Disorders

Carnitine Palmytoyltransferase Deficiency Type I (CPTI)
Carnitine Palmytoyltransferase Deficiency Type II (CPTII)
Carnitine/Acylcarnitine Translocase Deficiency (CACT)
Carnitine Uptake Deficiency (CUD)
Trifunctional Protein Deficiency
Very Long-chain Acyl-CoA Dehydrogenase Deficiency(VLCADD)
Long Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHADD)
Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)
3-HydroxyAcyl-CoA Dehydrogenase Deficiency (M/SCHADD)
Short Chain Acyl-CoA Dehydrogenase Deficiency (SCADD)
Glutaric Acidemia Type II (MADD)
Ethylmalonic Aciduria
2,4 Dienoyl CoA Reductase Deficiency

Organic Acid Disorders

Malonic Aciduria
Isovaleric Aciduria
Propionic Aciduria
Glutaric Aciduria Type I
Methylmalonic Aciduria
3-Ketothiolase Deficiency
3-Methylcrotonyl-CoA Carboxylase Deficiency
3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency
Multiple-CoA Carboxylase Deficiency
Maternal Vitamin B12 Deficiency
Isobutyryl CoA Dehydrogenase Deficiency
2-Methylbutyryl CoA Dehydrogenase Deficiency
2 Methyl-3 Hydroxy butyryl CoA Dehydrogenase Deficiency

Amino Acid Disorders

Phenylketonuria (PKU)
Maple Syrup Urine Disease (MSUD)
Homocystinuria
Tyrosinemia Type I, II , III
Argininosuccinic Aciduria
Citrullinemia
Argininemia
Histidinemia
Hyperornithinemia
Hyper/Hypomethioninemia
5-Oxoprolinuria (Pyroglutamic Aciduria)

Other Disorders :

X-Linked Adrenoleukodystrophy (X-ALD)
ADA-SCID, Adenosine Deaminase Deficiency (ADAD)
Argininosuccinic Acid Lyase Deficiency (ASA-LD)
Ornithine Transcarbamylase Deficiency (OTCD)
Carbamoyl Phosphate Synthase 1 (CPS-1)
N-Acetylglutamate Synthase (NAGS)

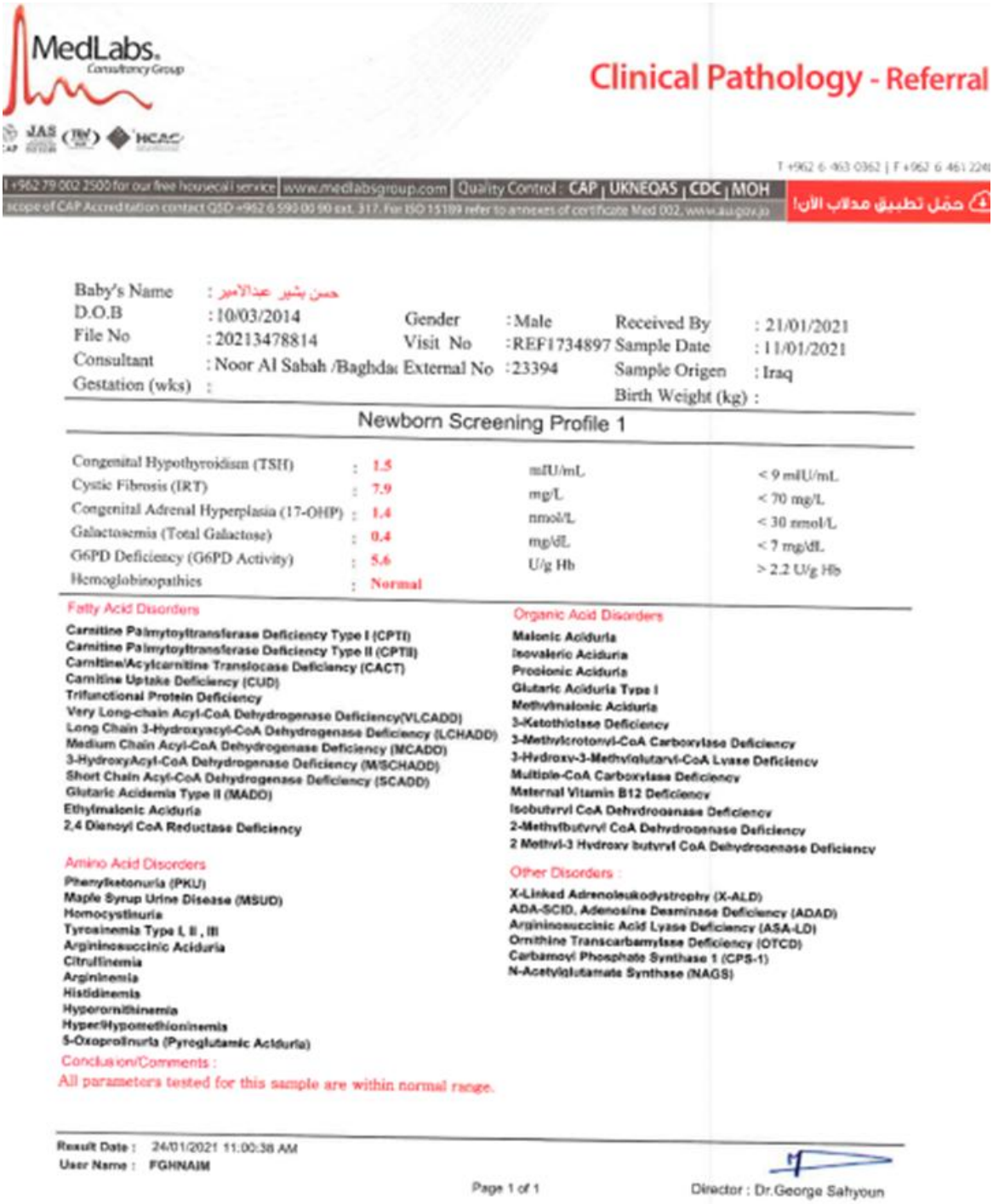
Conclusion/Comments :
G6PD enzyme activity was detected below the normal range.
A second sample is advised to confirm the result.
All other parameters tested for this sample are within normal range.

Result Date : 21/07/2020 01:16:27 PM
User Name : FGHNAIM

Page 1 of 1

Director : Dr.George Sahyoun

Figure 22 shows the result of patient with TMS



MedLabs. Consultancy Group

Clinical Pathology - Referral

T +962 6 463 0362 | F +962 6 461 224

+962 79 002 2500 for our free housecall service | www.medlabsgroup.com | Quality Control : CAP | UKNEQAS | CDC | MOH

scope of CAP Accreditation contact QSD +962 6 590 00 90 ext. 317. For ISO 15189 refer to annexes of certificate Med 002, www.sa.gov.jo

حمّل تطبيق مدلاب الآن!

Baby's Name	: حسن بشير عبدالامير				
D.O.B	: 10/03/2014	Gender	: Male	Received By	: 21/01/2021
File No	: 20213478814	Visit No	: REF1734897	Sample Date	: 11/01/2021
Consultant	: Noor Al Sabah /Baghdad	External No	: 23394	Sample Origen	: Iraq
Gestation (wks)	:			Birth Weight (kg)	:

Newborn Screening Profile 1

Congenital Hypothyroidism (TSH)	: 1.5	mIU/mL	< 9 mIU/mL
Cystic Fibrosis (IRT)	: 7.9	mg/L	< 70 mg/L
Congenital Adrenal Hyperplasia (17-OHP)	: 1.4	nmol/L	< 30 nmol/L
Galactosemia (Total Galactose)	: 0.4	mg/dL	< 7 mg/dL
G6PD Deficiency (G6PD Activity)	: 5.6	U/g Hb	> 2.2 U/g Hb
Hemoglobinopathies	: Normal		

Fatty Acid Disorders

Carnitine Palmytoyltransferase Deficiency Type I (CPTI)
Carnitine Palmytoyltransferase Deficiency Type II (CPTII)
Carnitine/Acylcarnitine Translocase Deficiency (CACT)
Carnitine Uptake Deficiency (CUD)
Trifunctional Protein Deficiency
Very Long-chain Acyl-CoA Dehydrogenase Deficiency(VLCADD)
Long Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHADD)
Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)
3-HydroxyAcyl-CoA Dehydrogenase Deficiency (M/SCHADD)
Short Chain Acyl-CoA Dehydrogenase Deficiency (SCADD)
Glutaric Acidemia Type II (MADD)
Ethylmalonic Aciduria
2,4 Dienoyl CoA Reductase Deficiency

Amino Acid Disorders

Phenylketonuria (PKU)
Maple Syrup Urine Disease (MSUD)
Homocystinuria
Tyrosinemia Type I, II , III
Argininosuccinic Aciduria
Citrullinemia
Argininemia
Histidinemia
Hyperornithinemia
Hyper/Hypomethioninemia
5-Oxoprolinuria (Pyroglutamic Aciduria)

Organic Acid Disorders

Malonic Aciduria
Isovaleric Aciduria
Propionic Aciduria
Glutaric Aciduria Type I
Methylmalonic Aciduria
3-Ketothiolase Deficiency
3-Methylcrotonyl-CoA Carboxylase Deficiency
3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency
Multiple-CoA Carboxylase Deficiency
Maternal Vitamin B12 Deficiency
Isobutyryl CoA Dehydrogenase Deficiency
2-Methylbutyryl CoA Dehydrogenase Deficiency
2 Methyl-3 Hydroxy butyryl CoA Dehydrogenase Deficiency

Other Disorders :

X-Linked Adrenoleukodystrophy (X-ALD)
ADA-SCID, Adenosine Deaminase Deficiency (ADAD)
Argininosuccinic Acid Lyase Deficiency (ASA-LD)
Ornithine Transcarbamylase Deficiency (OTCD)
Carbamoyl Phosphate Synthase 1 (CPS-1)
N-Acetylglutamate Synthase (NAGS)

Conclusion/Comments :

All parameters tested for this sample are within normal range.

Result Date : 24/01/2021 11:00:38 AM
User Name : FGHNAJM

Page 1 of 1

Director : Dr.George Sahyoun

Figure 23 shows the result of patient with TMS

MedLabs. Consultancy Group

Clinical Pathology - Referra

T +962 6 493 0362 | F +962 6 461 2

+962 79 002 2500 for our free house call service | www.medlabsgroup.com | Quality Control : CAP | UKNEQAS | CDC | MOH

In scope of CAP Accreditation (contact QSD +962 6 580 00 90 ext. 317. For ISO 15189 refer to annexes of certificate Med 002, www.sa.gov.jo)

حمّل تطبيق مدلاب الآن!

Baby's Name	: ترجس باسم محمد				
D.O.B	: 18/08/2020	Gender	: Female	Received By	: 24/11/2020
File No	: 20203415440	Visit No	: REF1615424	Sample Date	: 12/11/2020
Consultant	: Noor Al Sabah /Baghdad	External No	: 23362	Sample Origen	: Iraq
Gestation (wks)	:			Birth Weight (kg)	:

Newborn Screening Profile 1

Congenital Hypothyroidism (TSH)	: 5.3	mIU/mL	< 9 mIU/mL
Cystic Fibrosis (IRT)	: 19.2	mg/L	< 70 mg/L
Congenital Adrenal Hyperplasia (17-OHP)	: 0.6	nmol/L	< 30 nmol/L
Galactosemia (Total Galactose)	: 2.6	mg/dL	< 7 mg/dL
G6PD Deficiency (G6PD Activity)	: 3.6	U/g Hb	> 2.2 U/g Hb
Hemoglobinopathies	: Normal		

Fatty Acid Disorders

Carnitine Palmytoyltransferase Deficiency Type I (CPTI)
Carnitine Palmytoyltransferase Deficiency Type II (CPTII)
Carnitine/Acylcarnitine Translocase Deficiency (CACT)
Carnitine Uptake Deficiency (CUD)
Trifunctional Protein Deficiency
Very Long-chain Acyl-CoA Dehydrogenase Deficiency(VLCADD)
Long Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHADD)
Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)
3-HydroxyAcyl-CoA Dehydrogenase Deficiency (M/SCHADD)
Short Chain Acyl-CoA Dehydrogenase Deficiency (SCADD)
Glutaric Acidemia Type II (MADD)
Ethylmalonic Aciduria
2,4 Dienoyl CoA Reductase Deficiency

Organic Acid Disorders

Malonic Aciduria
Isovaleric Aciduria
Propionic Aciduria
Glutaric Aciduria Type I
Methylmalonic Aciduria
3-Ketothiolase Deficiency
3-Methylcrotonyl-CoA Carboxylase Deficiency
3-Hydroxy-3-Methylglutaryl-CoA Lyase Deficiency
Multiple-CoA Carboxylase Deficiency
Maternal Vitamin B12 Deficiency
Isobutyryl CoA Dehydrogenase Deficiency
2-Methylbutyryl CoA Dehydrogenase Deficiency
2 Methyl-3 Hydroxy butyryl CoA Dehydrogenase Deficiency

Amino Acid Disorders

Phenylketonuria (PKU)
Maple Syrup Urine Disease (MSUD)
Homocystinuria
Tyrosinemia Type I, II , III
Argininosuccinic Aciduria
Citrullinemia
Argininemia
Histidinemia
Hyperornithinemia
Hyper/Hypomethioninemia
5-Oxoprolinuria (Pyroglutamic Aciduria)

Other Disorders :

X-Linked Adrenoleukodystrophy (X-ALD)
ADA-SCID, Adenosine Deaminase Deficiency (ADAD)
Argininosuccinic Acid Lyase Deficiency (ASA-LD)
Ornithine Transcarbamylase Deficiency (OTCD)
Carbamoyl Phosphate Synthase 1 (CPS-1)
N-Acetylglutamate Synthase (NAGS)

Conclusion/Comments
All parameters tested for this sample are within normal range.

Result Date : 25/11/2020 12:05:19 PM
User Name : FGHNAIM

Page 1 of 1

Director : Dr.George Sahyoun

الخلاصة:

يتم تنظيم عملية التمثيل الغذائي في مسارات حيث يتم تحويل المكونات من خلال عمل مختلف الإنزيمات التي تحفز تحول الركائز (البناء او الهدم). عندما لا يعمل واحد أو أكثر من هذه الإنزيمات بشكل صحيح، يمكن أن تحدث اضطرابات التمثيل الغذائي. يتم توريث معظم الأخطاء الفطرية في التمثيل الغذائي بطريقة وراثية متنحية. يقدر معدل حدوث الأخطاء الخلقية في التمثيل الغذائي من 1: 800 إلى 1: 1000 ولادة حية ، على الرغم من أن الحدوث ربما يكون أعلى بكثير نظرًا للصعوبات في التشخيص السريري والقيود في الاختبارات التشخيصية كانت نسبة حدوث وانتشار الأخطاء الفطرية في التمثيل الغذائي تختلف في بلدان مختلفة. يعتمد التصنيف على عملية التمثيل الغذائي المتأثرة أو الإنزيمات المشاركة في إنتاج المواد السامة مثل الاضطرابات الفردية للأحماض الأمينية، واضطرابات التمثيل الغذائي للكربوهيدرات، واضطراب التمثيل الغذائي للدهون، واضطرابات ايض البيور ينات والبيريميدين.

كان الهدف هو إدخال طريقة TLC لفصل الأحماض الأمينية القياسية التي يمكن أن تكون فعالة في الكشف عن اضطرابات الأحماض الأمينية من خلال إنتاج استراتيجيات يمكن تمييزها على أساس ألواح طبقه رقيقه اللوني من هلام السيليكا. تم اختيار العينات الدراسه الحاليه من المرضى الموجودين في وحدة الأمراض النادرة بمستشفى كربلاء التعليمي للأطفال. ، وتم تحليل 20 حمض أميني قياسي بواسطة الطبقة الرقيقة اللوني (10 مرات لكل حمض أميني). بعد ذلك تم فصل الأحماض الأمينية في المصل والبول لمجموعه المرضى، وشملت الدراسة 56 عينة تتراوح أعمارهم من 7 أيام إلى 15 سنة. تم تقسيم عينات المرضى على النحو التالي: حديثي الولادة الذين تقل أعمارهم عن 1 شهر كانوا 7، حديثي الولادة الذين تتراوح أعمارهم بين 2-12 شهرًا وعددهم 39، والأطفال الذين تزيد أعمارهم عن سنة 10، والإناث 22، والذكور 34، وأظهرت دراستنا أن 41٪ من كان مرضى الأخطاء الفطرية في التمثيل الغذائي المشتبه بهم يعانون من علامات اليرقان، و 32٪، 29٪، 18٪ ، 16٪ ، 5٪ ، 5٪ مع انتفاخ البطن والقيء والإسهال وحموضه الدم والجفاف وتضخم الكبد على التوالي. أظهرت النتائج أن حوالي 50٪، 41٪، 39٪، 36٪، 18٪ من مرضى الأخطاء الفطرية في التمثيل الغذائي المشتبه بهم يعانون من نقص التوتر والتشنج والتأخر الذهني ونوبات الصرع والخمول على التوالي. تم إجراء قياس الأحماض الأمينية على الطبقة الرقيقة اللوني بناءً على استراتيجيات مختلفة: تم إنشاء نتائج الدراسة على شكل استراتيجيات مختلفة تظهر إثباتًا

جيدًا لقياس الاحماض الأمينية في جميع أنحاء عامل الاحتفاظ (المتوسط و / أو النطاق)، أدى عامل الاحتفاظ الحسابي الى تقسيم الاحماض الأمينية في 6 مجاميع وفقًا لذلك. كما تم فصل الاحماض الأمينية القياسية بناءً على إنتاج ألوان / أشكال مختلفة التي يمكن تمييزها. تساعد الطرق الثلاثة المذكورة أعلاه في تحديد الأحماض الأمينية في عينات البول والمصل. تم تحديد عن أن ثلث الأخطاء الفطرية في التمثيل الغذائي كان من اضطرابات الأحماض الأمينية، ويجب تشخيص هذه الاضطرابات وعلاجها في أقرب وقت ممكن لمنع المضاعفات السيئة. بشكل عام، للحصول على التشخيص، يجب إجراء قياس الطيف الكتلي الترادفي &سائل فاصل للون عالي الكفاءة للمرضى المشتبه بهم إذا كان لديهم اضطراب الأحماض الأمينية، وكلا الطريقتين مكلفتين وتستغرق وقتًا طويلاً ومتوفرة فقط في مواقع قليلة في العراق. من أجل تجاوز هذه الآثار الضارة، اقترحت هذه الدراسة البدء بالطبقة الرقيقة اللوني (سهل، وغير مكلف، وفعال من حيث الوقت) يمكن أن يساعد في فحص المرضى المشتبه بهم الذين يعانون من اضطرابات الأحماض الأمينية، ثم يمكن استخدام الطرق الأكثر تحديدًا وحساسية (قياس الطيف الكتلي الترادفي & سائل فاصل للون عالي الكفاءة) فقط لإثبات التشخيص.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء / كلية الطب
فرع الكيمياء والكيمياء الحياتية

فائدة الطبقة الرقيقة اللوني للكشف المبكر عن اضطراب الأحماض الأمينية

رسالة مقدمة الى مجلس كلية الطب / جامعة كربلاء كجزء من متطلبات

نيل شهادة الماجستير في الكيمياء السريرية

من قبل

منى علي حسين

(بكالوريوس طب وجراحه بيطريه 2017)

اشراف

ا.م.د رنا مجيد حميد
دكتوراه كيمياء حياتيه سريرية
كليه الطب –جامعه كربلاء

د. رياض عبد الرسول حنيوه
بورد كيمياء مرضيه
مستشفى الحسين التعليمي –كربلاء

(1443) هجري

(2021) ميلادي