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INBORN ERRORS OF AMINO ACID AND CARBOHYDRATE METABOLISM

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ABSTRACT... nafeeskhan50@yahoo.com **Objectives:** This study was conducted with two objects in mind. Firstly, to screen children who were under two years of age for detection of inborn errors of Amino Acid and Carbohydrate metabolism. Secondly, a group of cases of proved mental deficiency were screened to find out whether the inborn errors of Amino Acid and Carbohydrate metabolism are a significant contributory factor to the mental deficiency in Pakistan. **Setting:** Department of Pathology, PGMI/ KEMC/ Mayo Hospital Lahore. **Material and methods:** In this study, two groups of subjects were investigated systematically to detect inborn errors of amino acid and carbohydrate metabolism with particular reference to Alcaptonuria, Phenylketonuria, Galactosemia, FruSosuria and pentosuria. In group I - 2000 children, under 2 years of age, mostly newborns, were randomly selected for this study from Pediatrics department of various hospitals of Lahore. Group II - Includes 30 cases of mental deficiency of various ages referred by the psychiatrists or pediatricians for verifying whether any inborn error of Amino Acid or Carbohydrates metabolism is present or not. **Method:** Chemical screening tests along with one dimensional descending paper chromatography and thin layer chromatography (TLC) were employed to detect metabolic errors. **Results:** In group I, one case of alcaptonuria was detected In group I!, Three cases of a specific generalized aminoaciduria occurring in a single family were detected. **Conclusion:** This study indicates that inborn errors of metabolism also exist in Pakistan. It has also sorted out a reliable scheme of screening and detection of these disorders suited to our socio-economic.environment. The importance of early suspicion of these disorders and a timely diagnosis during preventable stage has been stressed and the need of a national screening programme highlighted.

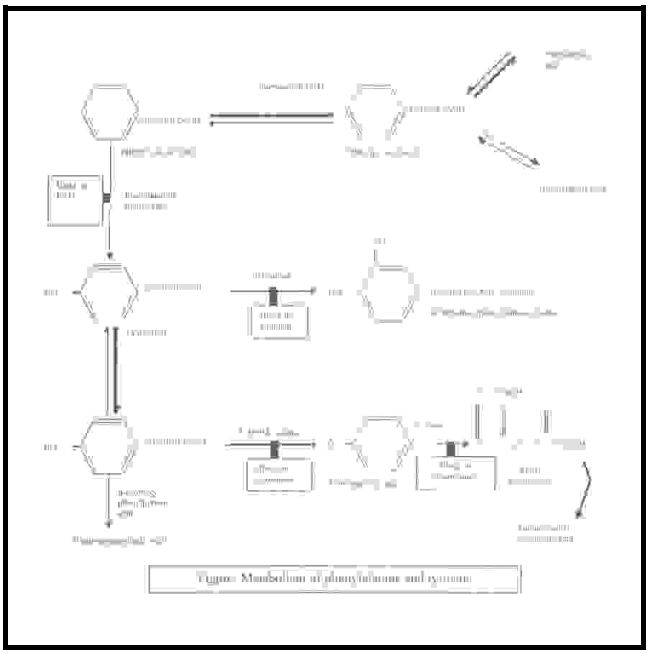
Key words: Inborn errors of metabolism, screening programme, early detection and treatment, chromatography, phenylketonuria, alcaptonuria, galactosemia.

INTRODUCTION

Around the turn of the twentieth century Sir Archibald Garrod[1] (1908) drew attention to the inborn errors of metabolism, rare disorders each characterized by a specific block in biochemical pathway and resulting in the disposal by some alternative route of a metabolite immediately preceding that metabolic block. He then postulated that these abnormalities were due to

inheritable enzyme deficiencies. Since then a large number of genetic disorders have been recognized in which a defect of amino acid or carbohydrate metabolism is coupled with neurologic symptoms, most commonly, mental retardation. It has also been recognized that while some of these abnormalities, such as pentosuria. arc without injurious affects, others including Phenylketonuria (PKU) are definitely harmful[2]. PK.U is one of these disorders which has been extensively studied so far[3-9].

PKU is a disorder of phenylalanine metabolism in which the enzyme responsible for the conversion of phenylalanine to tyrosine, phenylalanine hydroxylase, is completely missing[3,12]. Because of the defect, phenylalanine accumulates throughout the body and is converted to phenylpyruric acid and other metabolites: phenyl lactic acid, phenyl acetic acid, phenylacetylglutamine and o-hydroxyphenylacetic acid (Figure 1).



These are not abnormal metabolites; rather they are normal metabolites that occur in very abnormal amounts in this disorder[4]. Untreated phenylketonurics present with mental retardation, seizure activity, pigment dilution, eczema, cataract and microcephaly[4,5,6]. According to Knox (1978)[10], one third is unable to walk and two thirds are unable to talk. None of the symptoms are specific but it may be that these will be found to be more frequent in true phenylketonurics.

The incidence of PKU varies from 1 in 4500 in Ireland[11] to 1 in 11,767 in USA[13]. In 2006, NIH[14] reported that incidence in the U.S, Britain, and most of Western Europe is between 1 in 11,000 and 1 in 15,000 births. In Asian populations, PKU is rare and prevalence figures range from approximately 1:16,500 in China to 1:120,000 in Japan[14,38].

In the US, the incidence of congenital hypothyroidism, as detected through newborn screening is approximately 1 per 4000. Data from countries with well established newborn screening programs indicate an incidence of congenital hypothyroidism of 1 per 3000-4000. Some countries with more recently established screening programs have slightly higher incidences. Some of the highest incidences (1 in 1400 to 1 in 2000) have been reported from various locations in the Middle East[15,16,17]. Studies conducted since 1979 show that 1 of every 7,500 live births will have some form of galactosemia[18]. It is also estimated that 1 of every 40 people is a carrier of this defective gene[18,19,20]. Alkaptonuria affects one in 250,000 to one million people worldwide[21]. In US, the incidence is 1 case per 4 million populations. This disease is unusually common in Slovakia where incidence is 1 in 19,000[21,22].

It is now generally accepted that if the PKU is promptly diagnosed at birth, a low phenylalanine diet will permit the infants brain to develop normally[23]. Thus according to Smith & Wolff (1974),[24] extreme urgency in prompt and accurate diagnosis is indicated if mental retardation is to be avoided.

Bickel (1971),[25] Holtzman et al (1986)[26], Bickel (1996)[27] suggested that early detection and treatment of hereditary metabolic diseases must be carried out on large scale. All newborns should be tested for PKU, maple syrup urine disease, homocystinuria and galactosemia during the early days of life.

In 1974, Holtzman et al.[28] reported that about 90% of all North American infants are screened for metabolic disorders of one form or another shortly after birth. By 1975, routine newborn screening for PKU became mandatory in 43 states of North America and in many parts of the world there is a similar trend towards early detection of inherited biochemical disorders[29]. In April 2006, NIH[14] reported that, all American states test for PKU and for congenital hypothyroidism, while 42 test for sickle-cell anemia, 38 for galactosemia and for additional metabolic disorders as well.

This trend has gained great momentum in recent years, because it has been assumed that discovery of such abnormalities in the newborn period of life will allow the physician to initiate treatment so that the potential harm which would result from biochemical imbalance can be offset. Virtually, today all newborns are tested for PKU in every American state, Canada, Australia, New Zealand. Japan, the nations of Western and most of Eastern Europe, and many other countries throughout the world. At relatively low cost, it has prevented mental retardation in thousands of infants worldwide[14,38,39]. It is a significant achievement that these individuals and their families have been spared the devastating effects of the PKU and other inborn disorders.

The primary role of screening thus appears to be preventive. But in Pakistan, no such steps have so far been taken on national level for early detection and treatment of these inherited metabolic disorders in newborn babies. The screening methods can also be used in this country to detect metabolic abnormalities in newborn babies. This will not only provide early diagnosis but also will provide a guideline for the management of such cases and will help to develop them normally. The present study was carried out with two objects in mind:

Firstly, to screen urine of children who were under two years of age for detection of inborn errors of amino acid and carbohydrate metabolism with particular reference to the 1-Alkaptonuria, 2- Phenylketonuria (PKU), 3- Galactosemia, 4, Pentosuria and 5- Fructosuria.

Secondly, it was aimed at finding out whether the above-mentioned inborn errors of metabolism are a significant contributory factor to the mental deficiency in this country. For this, a group of clinically proved cases of mental deficiency was screened for these disorders.

MATERIAL AND METHODS

In this study, two groups of subjects were investigated systematically to detect inborn errors of amino acid and carbohydrate metabolism with particular reference to the following:

1. Alkaptonuria
2. Phenylketonuria (PKU)
3. Galactosemia
4. Fructosuria
5. Pentosuria

Group-I: Two thousand children of under two-year age were randomly selected for this study from pediatric departments of various Lahore hospitals (Table-I).

Table-I. Division of group I children into various sub groups according to age

Sub group	Age (months)	Sex		Total both sexes
		Male	Female	
1	0-1	424	286	710
2	1-3	205	121	326
3	3-6	114	103	217
4	6-12	168	107	275
5	12-24	283	189	472
Grand Total		1194	806	2000

Group-II: Thirty cases of mental deficiency of various ages referred by the psychiatrists or pediatricians for verifying if any of the above mentioned inborn errors of metabolism is present or not (Table-II).

Table-II. Division of group II (mental deficiency) case into two sub-groups according to age.				
Sub group	Age (years)	Sex		Total both sexes
		Male	Female	
1	1-5	08	11	19
2	5-10	05	06	11
Total		13	17	30

Chemical screening tests (FeC13 and Benedict's tests) and one dimensional descending paper chromatography and thin layer chromatography (TLC) were employed to detect metabolic errors[30].

Early morning urine specimens from both these groups were screened for the detection of above mentioned disorders and in those giving a positive result, quantitation of various substances in blood or urine was also done as indicated.

RESULTS

The results of this screening program, on urine specimens of 2,030 children are as follows:

Group-I: Out of 2,000 cases (1194 males and 806 females) only one female child had alkaptonuria. The elder sister of this child examined as part of the family study also showed the presence of this disorder. There were no cases of PKU, galactosemia, fructosuria, pentosuria or any other aminoaciduria or melliturıa (Table-III).

Group-II: Out of 30 cases aged 1-10 years (13 males and 17 females); there were three cases of generalized aminoaciduria belonging to one family. The aminoaciduria pattern of this family did not fit into any of the recognized aminoacidurias so far reported in the literature. But it is definitely related to the mental deficiency in the family because all other close members of the family that were studied had no mental deficiency nor had they aminoaciduria. So this appears to be a new syndrome. (Table-IV).

Table-III. Results of urine screening in 2000 random hospitalized under 2 years children.		
Category	Disorder	No. of cases
Aminoaciduria	Alkaptonuria	01
	Others	Nil
	Total	01
Mellituria		Nil
Normal		1999
Grand Total		2000

Table-IV. Results of urine screening in 30 cases of mental deficiency.		
Category	Disorder	No. of cases
Aminoaciduria	Generalized Aminoaciduria	03
	Others	Nil
	Total	03
Mellituria		Nil
Total with metabolic error		03
Without aminoaciduria or mellituria		27
Grand Total		30

DISCUSSION

This documented study on inborn errors of metabolism in the Punjab (Pakistan) gives the results of a thorough biochemical screening by means of well-proved methods.

The first part of the study covers 2,000 random cases aged less than 2 years in which one case of alkaptonuria

was detected.

The second part of the study reports results of screening tests carried out on 30 mentally deficient cases and in this -group three cases (belonging to one family) of an obscure generalized aminoaciduria were detected. A search of literature did not reveal that this pattern of aminoaciduria have ever been reported previously. The fact that it occurred in three members of a single family and all three were having an identical pattern proves its hereditary origin.

The study shows that the problem of inborn errors of metabolism does exist in this province (Punjab). We cannot pick cases of any disease unless we are conscious of its existence and suspect it. And this study has brought us to the conclusion that classical inborn errors of metabolism like alkaptonuria do occur here and we need to know their nature and incidence by means of national survey.

In 1970, Sridhara et al.[31] found 5 phenylketonurics in 414 cases of mental deficiency examined in Banglore, India. In Kuwait, Teebi et al.[32] found 7 cases of PKU among 451 institutionalized mentally retarded persons (1.9%).

The need of this survey is indicated by the fact that the modern medicine has made it possible to prevent and manage this class of disorders. Over the past few decades it has become apparent that a number of inborn errors of metabolism are associated with clinical abnormalities, particularly mental retardation and that in several of these disorders, the consequences may be prevented by early institution of therapy[2,39]. Foremost among the treatable disorders is PKU[24,26,33,35,36,40], but also included are maple syrup urine disease, homocystinuria and galactosemia[25,39]. Ideally, galactosemia and maple syrup urine disease should be treated from birth and PKU before 3 months. This has led to the view that all the newborn babies should be screened for hereditary metabolic disorders, so that they could be put on treatment before the disorder causes irreversible clinical damage[2,39].

At relatively low cost, it has prevented mental retardation in thousands of infants worldwide. It is a significant achievement that these individuals and their families have been spared the devastating effects of the phenylketonuria and other inborn disorders[37-40].

Infact, routine newborn screening for PKU and congenital hypothyroidism has become mandatory in all states of USA and has spread widely in Europe and other areas of the world. A number of these screening programs are now testing for many metabolic disorders in addition to PKU. These screening programs have yielded invaluable and otherwise unobtainable information concerning the incidence and characteristics of certain metabolic disorders among the general population[14,28,29,34]. Until now, diagnosis of metabolic errors was made by the positive findings with the chemical tests. But in view of the other disorders showing false positive with chemical tests, diagnosis of metabolic errors is now confirmed with chromatographic methods. Therefore, in addition to chemical tests, one dimensional descending paper chromatography and TLC was used in this study for the detection of metabolic errors[30] and proved quite satisfactory. The chromatography has the advantages of being cheap and capable of handling a large number of individual samples simultaneously on a single sheet. Thus the methodology employed in this pilot study, which is both cheap and reliable for detecting the maximum number of cases can easily be adopted for the national survey program.

CONCLUSION

This study indicates that inborn errors of metabolism also exist in Pakistan. It has also sorted out a reliable scheme of screening and detection of these disorders suited to our socioeconomic environment. The screening program can yield invaluable and otherwise unobtainable information concerning the incidence and characteristics of certain metabolic disorders among the general population.

Early detection is important for prevention and treatment of multiple systems. The detection of these disorders is not without benefit for the affected family and the

individual as the administration of un-necessary drugs can be avoided. In addition to being cheap and reliable, chromatography is of greatest value when both disorders of amino acid and carbohydrate metabolism are to be detected simultaneously.

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