TEN YEARS EXPERIENCE OF A METABOLIC INVESTIGATION LABORATORY IN TURKEY

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SUMMARY

Purpose: A high prevalence of inherited metabolic diseases is present in Turkey, at least in part; due to consanguinous marriages. We screened a group of selected infants and children with motor and mental retardation or having risk factors for metabolic disorders. **Methods:** We screened approximately 20.000 cases since 1991 in our laboratory. As a standard rule, the following methods were employed: Initially paper chromatography, drop, dip and spot tests, and later high performance liquid chromatography and more recently gas chromatography. Rewets were confirmed by enzymatic analysis when necessary. **Results:** A total of 146 metabolic disease cases were diagnosed and classified into 22 various diseases. The proportion of positive diagnosis was about 1 in 142. **Conclusion:** Incidence of inherited metabolic disorders in our country will probably increase in new future with administration new laboratory techniques.

Key Words : *Metabolic Disease, Metabolic Investigation.*

INTRODUCTION

A high prevalence of inherited metabolic diseases is present in Turkey; at least in part, this is due to consanguinous marriages (1,2). Recent advances in the diagnosis and treatment of inborn errors of metabolism have substantially improved the prognosis for many of these conditions. Progress in diagnosis of such conditions has been closely connected with progress in laboratory methodology.

Selective screening for inborn errors of metabolism was started in 1950s by the personal interest of motivated clinicians and biochemists. Subsequently these intentions resulted with establishing small teams, mostly within universities, who were involved in the diagnosis and development of rational therapy of inherited metabolic diseases. In the 1970s, new analytical instrumentation and chromatographic and mass spectrometric techniques allowed the investigation of a wide range of disorders (3).

Today, the application of molecular biology is rapidly expanding the possibilities for diagnosis and our understanding of metabolic diseases (4,5).

In 1991, The Pediatric Metabolism Laboratory at the Gazi University Hospital Department of Pediatrics began screening for inborn errors of metabolism from a selected population. Specimens from individuals suspected of a metabolic disorder due to their clinical features or family history were sent from various hospitals in the city and the department itself.

PATIENTS AND METHODS

Investigations were performed on physical findings of patients with failure to thrive, developmental delay, seizures, failure to feed or persistent vomiting, prolonged jaundice, hypotonia or hypertonia, unusual urine odour, cataracts and a history of a relative with inherited metabolic disease.

Urine and blood as liquid samples were investigated. The samples were sent to the laboratory with a special form of clinical and laboratory finding characteristic of metabolic diseases. Urine and blood samples were investigated for amino acids by one or two-dimensional paper chromatography initially. Also some clinical diagnostic tests were made on each urine sample. These include the investigation of a reducing substance Benedict solution, Fe_3Cl test for phenylketonuria, cyanide nitroprusside test for cystinuria and/or homocystinuria, 2,4-dinitrophenylhydrasine test for ketonuria and spot test for methylmalonic acidemia (6). Positive results for reducing substance and cyanide nitroprusside tests were confirmed by sugar and cystine-homocystine chromatogaphies respectively. Abnormal results of any of these tests were confirmed by High Performance Liquid Chromatography (HPLC).

Urine samples of the patients suspected to have organic acidemia have been also examined by gas chromatography since 1998. (Table 1) Diagnosis was confirmed by enzymatic analysis in various centres in Europe when necessary.

RESULTS

Since 1991, a total of 20,698 selected cases have been screened. During this period, 146 cases were diagnosed and classified into 22 various metabolic diseases. (Table 2) The overall incidence of positive findings was about 1 in 142. (‰ 7)

DISCUSSION

Inborn errors of metabolism and other inherited disorders are common in Turkey, presumably because of the relatively high rates of consanguinous marriages. (%21) (1).

In the past, a small number of samples were analyzed for metabolic diseases. With growing experience and techniques, the number of specimens and the incidence of positive findings have increased gradually (7).

We started screening of the selected patients in our laboratory in 1990. Since that time, as mentioned before, 146 patients out of 20,698 have been found to have one of the 22 different metabolic diseases, with an incidence of 1 in 142. Among the diseases, cystinuria and phenylketonuria were the most prevalent. (%0.21 and %0.10 respectively).

In a prospective study carried out with 8572 cases aged 20 days - 20 years in Turkey, the diagnosis of cystinuria was confirmed in 7 with a prevalence of 1:2155 (8). In this study, patients were initially screened with the cyanide-nitroprusside test (CNP). CNP-positive patients were investigated by one or two dimensional paper chromatography. Also quantitative cystine determinations were made in the 24 hour urine samples of the patients. In another study, the incidence was found to be 1/2155 in elementary school students. These results indicate that cystinuria is not rare among Turkish children with urinary stones and that screening every child with urolithiasis should be encouraged.

The incidence of phenylketonuria is as high as 1/4500 among Turkish children (9). An attempt to perform a nation wide population screening program for phenylketonuria by the Guthrie test is now being made in Turkey and the results of this program could be expected to reveal a large number of additional cases (2). There is also an urgent need to educate the public about the risk and bed outcomes of consanguinous marriages.

In another center in Turkey, 6292 patients were selectively screened and 70 were found to have hyperphenylalaninaemia, 8 biotinidase deficiency, 7 methylmalonic aciduria, 7 homocystinuria, 7 cystinuria, 7 maple syrup

Table 1: Selective screening program for the detection of inborn errors of metabolism in Gazi University Pediatric Metabolism Laboratory.

SCREENING TEST	MATERIAL
One and/or two dimensional paper chromatography	Urine and blood
Screening tests	Urine
Reducing substance	
Fe_3Cl test	
Cyanide nitroprusside test	
Dinitrophenylhydrasine test	
Spot test for methylmalonic acid	
HPLC	Urine and blood
Gas chromatography	Urine

Table 2: Results of the screening programme of Gazi University Pediatric Metabolism Laboratory for the previous 9 years.

DISORDER	NUMBER OF PATIENTS	PREVALENCE (%)
Cystinuria	45	0.21
Phenylketonuria	22	0.1
Mucopolysaccharidoses	16	0.077
Glycogen storage disease	10	0.048
Homocystinuria	10	0.048
Galactosaemia	8	0.038
Transient hyperammonaemia	7	0.033
Canavan disease	4	0.019
Maple syrup urine disease	4	0.019
Benign fructosuria	3	0.014
Beta-kethothiolase deficiency	2	0.0096
Congenital lactic acidosis	2	0.0096
Cystinosis	2	0.0096
Lysinuric protein intolerance	2	0.0096
Tyrosinaemia	2	0.0096
Alkaptonuria	1	0.0048
Blue diaper syndrome	1	0.0048
Glutaric aciduria type II	1	0.0048
Hereditary fructose intolerance	1	0.0048
Isolated renal glycosuria	1	0.0048
Methylmalonic acidemia	1	0.0048
Porphyria	1	0.0048

urine disease, 7 galactosaemia, 4 lysinuric protein intolerance, 7 hereditary fructose intolerance, 7 primary lactic acidaemia, 6 urea cycle defect and 12 alcaptonuria (10).

Urine samples from selected patients have also been analysed by gas chromatography in the laboratory for the last year and one patient diagnosed to have beta-kethothiolase deficiency was hospitalised because of diarrhoea and metabolic acidosis and could easily have been been missed if this analysis had not been performed. Also 3 patients were found to have Canavan Disease by this technique. Before this method was introduced in our laboratory, the total number of patients found to have organic acidemia was only 5 and all the diagnoses were made in various centres of Europe. In a retrospective study done in Turkey, 186 patients were found to have organic aciduria (%22) out of 844 and 47 patients were diagnosed to have organic academia with gas chromatography mass spectrophotometry (11). The proportion of positive diagnosis will probably increase with such laboratory techniques.

For the investigation of metabolic diseases, well-equipped biochemical genetic laboratories are also needed, but they can not solve the whole problem. Good pre- and postgraduate training of physicians in the field of metabolic diseases, good cooperation between the referring physician and the laboratory and a comprehensive investigation of a broad spectrum

of metabolic disorders are equally important.

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