Screening for inborn errors of metabolism among mentally retarded patients

Outcome of a survey at the Witrand Care and Rehabilitation Centre

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Summary

The prevalence of different types of inborn errors of metabolism among the mentally retarded patients at the Witrand Care and Rehabilitation Centre, were determined by means of a biochemical screening survey. These results are compared with those of other surveys in South Africa and abroad. One important result points to substantial differences in the recorded incidences of metabolic defects between surveys. This observation could partially be due to significant differences between the different studies in terms of methodology employed and sampling procedures. The questions raised in this regard are documented and discussed.

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A national genetic programme aimed at preventing or coping with the problems of congenital and hereditary disorders was officially instituted for the RSA in 1977. An important part of the programme concerns the establishment of a co-ordinated and comprehensive genetic service in this country, available for every individual irrespective of community status or population group.

One of the means of achieving the objective of prevention is identification of high-risk families or groups of individuals, *inter alia* by way of screening tests, to provide them with the necessary counselling and to inform them about facilities at their disposal within the framework of a comprehensive genetic service. A special category of high-risk individuals qualifying for this service are the pupils at special schools as well as the residents of care and rehabilitation centres in the RSA. A genetic screening programme was instituted to detect cytogenetic and biochemical disorders among these residents. The methodological background and rationale for the implementation of a comprehensive genetic service for the severely mentally retarded has been outlined in a separate contribution.[1]

We now report on the biochemical screening tests used for the detection of the well-described inborn disorders of metabolism associated with mental retardation, as well as on the results obtained at the Witrand Care and Rehabilitation Centre at Potchefstroom, Transvaal.

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Patients

When the biochemical screening programme was initiated at Witrand Centre, very little information on the aetiology of the mental handicap of the patients was available. Moreover, for a substantial number the family history was fragmentary or even totally lacking. It was therefore agreed that all the patients at the institute would form the clinical material for the survey.

The age distribution of the patients in the Witrand Centre is shown in Fig. 1. Only 17% are under 15 years of age, and 50% are older than 33 years. These figures are approximates for males and females, although male patients tend to be somewhat younger. The oldest patient is a woman aged 84 years.

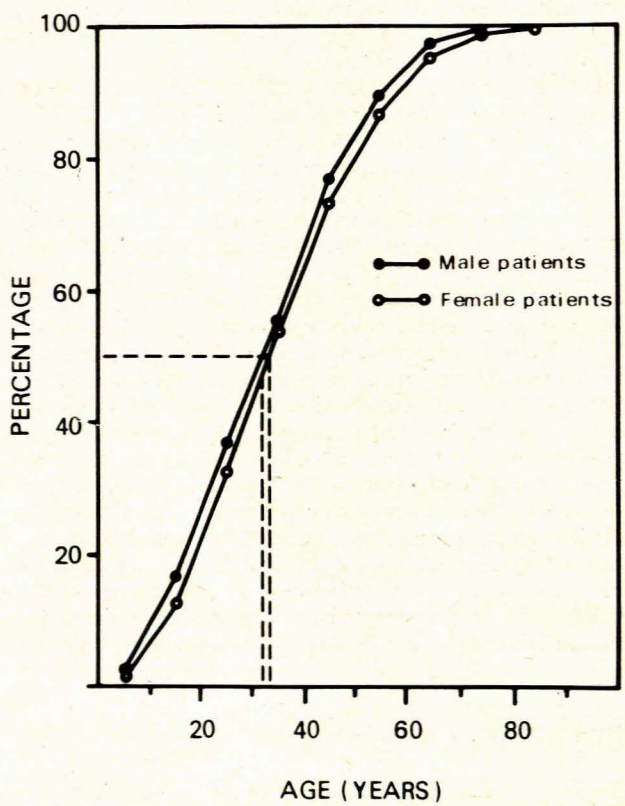

Fig. 1. Cumulative percentage of patients in the Witrand Institute, according to age.

Screening procedure

Specimens of early morning urine were sent by courier to the screening laboratory at the Department of Biochemistry, Potchefstroom University for CHE, and analysed immediately upon arrival. The medication taken by each patient was recorded for all specimens involved. The specimens were subjected to a battery of simple quantitative tests compiled to identify a sufficiently wide range of inborn errors of metabolism.

Phenylpyruvic acid was detected by Phenistix test strips (Ames) and the ferric chloride test.[2] Ketones and keto-acids were detected by the formation of dinitrophenylhydrazones upon addition of 2,4-dinitrophenylhydrazine to the urine containing these catabolites.[3] Reducing substances were detected by the Benedict test.[4] A persistent positive reaction (three independent determinations) was followed by one-dimensional chromatography to identify the carbohydrate if present.

Sulphur-containing amino acids were detected by the sodium cyanide-nitroprusside test[4] and tyrosine catabolites by the nitroso-naphthol test.[4] Histidine was tested for by the cuprizone test developed by Gerber and Gerber,[5] and mucopolysaccharides were tested for by the cetyltrimethylammonium bromide turbidity method[2] as well as the acid albumin turbidity test.[6] Amino acids present in excess were detected by two-dimensional chromatography on thin-layer plates.

For phenylalanine loading tests, the patients received L-phenylalanine 200 mg/kg body mass. Serum phenylalanine values were determined according to the method of Henry *et al.*[7] and tyrosine values according to the fluorometric method of Ambrose *et al.*[8] The amount of *p*-hydroxyphenolic acids excreted in the urine during the phenylalanine loading test was determined according to the method of Mienie[9] based on the principle of the α-nitroso-ß-naphthol test.[4]

Results

A total of 1 568 patients were screened for biochemical defects. The first results revealed that approximately 10% showed indications of biochemical disturbances. After repeated examination it appeared that the suspected defects were of a transient nature and mostly of no metabolic significance. Only 20 patients (1,3%) had consistent metabolic abnormalities as judged from the outcome of the screening tests. The syndromes associated with these patients are summarized in Table I, and are compared with results of similar surveys reported for Belfast/Northern Ireland[10] and Dublin,[11] Bangalore,[12] Michigan[13] and the Alexandra Institute, Cape Town.[14] Our findings, as well as those obtained for the Alexandra Institute, differ from those obtained in the other surveys in a number of respects, one being the prevalence of phenylketonuria. In the Witrand Centre only 2 patients (0,13%) and in the Alexandra Institute only 3 (0,28%) suffered from this disease. This is far less than the number of cases reported for Dublin, Belfast and Michigan. The figure reported for the Bangalore survey (0,83%) is higher than for the two South African institutions, and it indicates that phenylketonuria is probably less common in India than in Western Europe. This is also supported by results from a survey specifically associated with phenylketonuria among the mentally retarded in India.[15] The prevalence of phenylketonuria among the South African Indian population as well as in other population groups is still unknown, but it is likely to be lower than that for the White population group.

A second point of difference is the low overall prevalence of metabolic diseases at the Witrand Centre (1,28%) compared with other institutions. Only for the Alexandra Institute was a figure obtained (0,55%) which is even lower than that for Witrand.

It should be pointed out, however, that the data summarized for the various institutions are not always strictly comparable, because of the use of different criteria. The Bangalore group, for example, consisted of patients below the age of 15 years, and all had a low development quotient. Similar criteria were also used to a certain extent in the Northern Ireland survey. The Michigan group consisted of all patients aged 8 years and under and others of all ages whom the staff physicians had selected on the basis that they might be suffering from a genetic disorder. In contrast, the population group in the present study consisted of all the patients in the institution. A considerable number of these patients have since been shown to suffer from chromosomal defects (results in preparation). These patients would now be excluded from a programme of biochemical screening such as the Belfast, Dublin and Michigan studies.

The surveys also differ as regards methodology. Some of the investigators[14] used only fresh morning urine samples, as we did, while in the Dublin survey urine was collected 1 or 2 hours after a meal containing protein, so as to ensure detection of any tendency to amino-aciduria. It should be noted, however, that the prevalence of mild generalized amino-aciduria was not higher in this institution (0,7%) than the figure reported for Belfast (1,3%). The figure for the Witrand Centre (0,77%) is very near to that reported for Dublin. No figures for generalized amino-acidurias are reported for the Bangalore, Michigan and Alexandra Institute surveys. It is of importance to note that amino-aciduria is not itself a significant disorder, but it may represent a secondary or minor feature associated with a metabolic abnormality.

A final point of difference between the results obtained from the surveys summarized in Table I is the observation that certain diseases predominate among particular groups. The high prevalence of the cystine-lysine pattern (0,9%) and hyperglycinuria (2,14%) in the Dublin group and of mucopolysaccharidoses (0,96%) in the Michigan group are the most notable examples.

Amino acid malabsorption syndrome

A unique case of a patient with an amino acid malabsorption syndrome was found in the present survey. This was detected as a result of persistently high excretion of tyrosine catabolites as shown by a positive nitroso-naphthol test. This was originally interpreted as indicating persistent hypertyrosinaemia,[16] but the plasma tyrosine level was within the normal range. Loading tests for phenylalanine indicated that the absorption of this amino acid was greatly impaired (Fig. 2, A). The low absorption of phenylalanine was accompanied by high excretion of *p*-hydroxyphenolic acids (Fig. 2, B), reaching peak values 16 hours after oral ingestion of the phenylalanine dose. This observation, together with the characterization of the *p*-hydroxyphenolic acids by thin-layer chromatography, indicates that the origin of the abnormal catabolites in the urine is not related to a metabolic defect, as suggested by the screening test, but originates from gut flora.

Details of these experiments and other aspects of this case will be published shortly, but the finding is noted here to indicate the importance of detailed analyses, complementary to the screening tests. This is essential to a screening programme.

Conclusions

Our results indicate that the prevalences of inborn disorders of metabolism at the Witrand Centre are of the same order of magnitude as those reported for another South African institution.[14] This might relate to genetic factors as well as to a great similarity in methodology. Despite some technical differences, the results for the Witrand and Alexandra institutes clearly show that the incidence of inborn disorders of metabolism among mentally retarded patients is distinctly lower than figures reported for other countries.

TABLE I. COMPARATIVE RESULTS OF BIOCHEMICAL SCREENING TESTS AT VARIOUS INSTITUTIONS FOR THE MENTALLY RETARDED

	Belfast[10] (2 920)†		Dublin[11] (3 324)		Bangalore[12] (1 480)		Michigan[13] (727)		Alexandra[14] (1 087)		Potchefstroom* (1 568)	
Syndrome	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Amino acid malabsorption											1	0,064
Argininosuccinicaciduria	2	0,069	1	0,030								
Cystine-lysine pattern			30	0,903								
Cystinuria							2	0,275	2	0,184		
Hartnup disease					2	0,135			1	0,092		
Histidinuria					1	0,068					2	0,128
Homocystinuria	10	0,343	3	0,090	3	0,203						
Hydroxyprolinaemia					1	0,068						
Hyperglycinuria	3	0,103	72	2,166								
Galactosuria	3	0,103	0									
Glucosuria			6	0,181							3	0,191
Lowe's syndrome	1	0,034										
Methylmalonicaciduria							2	0,275				
Mild generalized amino-aciduria	38	1,301	24	0,722							12	0,765
Miscellaneous amino-aciduria			10	0,301								
Mucopolysaccharidoses	3	0,103	18	0,542			7	0,963				
Multiple carboxylase deficiency							1	0,138				
Ornithine transcarbamylase deficiency							1	0,138				
Phenylketonuria	69	2,363	69	2,076	14	0,946	6	0,823	3	0,276	2	0,128
Propionic acidaemia							1	0,138				
Proteinuria			60	1,805								
Total	129	4,419	293	8,816	21	1,420	20	2,750	6	0,552	20	1,276

*The present study.
†The number of patients screened in each study is shown in parentheses.

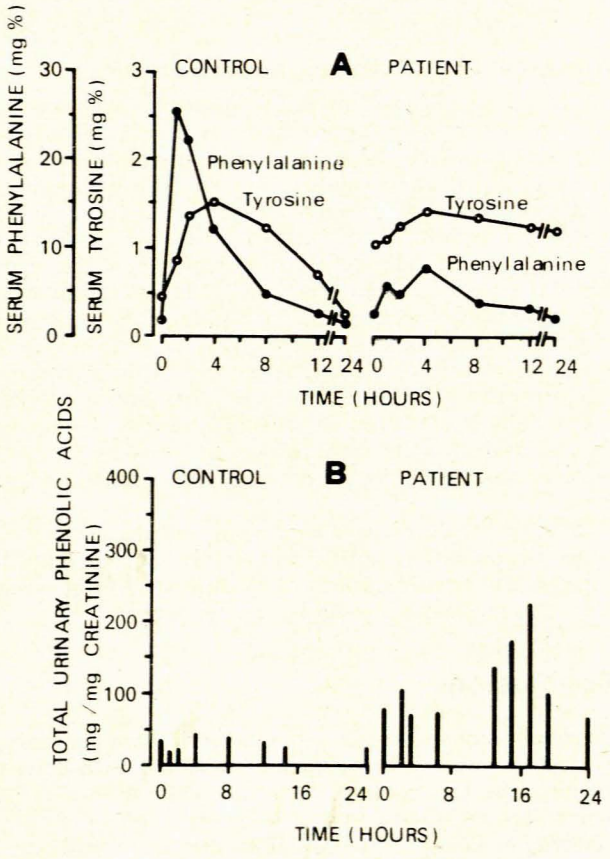

Fig. 2. A — loading tests for phenylalanine, and the formation of tyrosine from phenylalanine; B — excretion of *p*-hydroxyphenolic acids in the urine during the period of the phenylalanine loading test.

Our screening programme is designed to detect a variety of metabolic disorders. It would be of interest to compare the results in other population groups in the RSA. Investigations in this respect are being undertaken at present, and will be reported in the near future.

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