Screening for tyrosinaemia type I

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Abstract

Aims—To assess the incidence of tyrosinaemia type I in the West Midlands Region, and the value of current neonatal screening programmes for phenylketonuria (PKU) for its detection.

Methods—Retrospective study of results from the PKU neonatal screening programmes in Birmingham (using plasma amino acid chromatography) and in the rest of the West Midlands (using the Guthrie microbiological assay for blood spot phenylalanine) was carried out between January 1985 and March 1994. Patients with tyrosinaemia I born in the region during the same period were identified from a regional database of patients with confirmed inherited metabolic disease. The study was carried out in a specialist children's hospital; the regional centre in the West Midlands for neonatal screening and investigation of inborn errors, and a supraregional centre for liver transplantation and management of paediatric liver disease.

Results—Amino acid chromatography showed increased tyrosine in 447 of 145 444 neonates born in Birmingham; this was still increased at 6 weeks of age in six cases. Five had tyrosinaemia I; the sixth had tyrosinaemia type III. Two others in whom amino acid chromatography was considered normal have since presented with tyrosinaemia I. Outside Birmingham, 525 151 children were screened using the Guthrie test. Five have presented clinically with tyrosinaemia I; screening did not contribute to diagnosis in any case. The incidence of tyrosinaemia I was 1 in 20 791 live births within Birmingham and 1 in 105 037 outside. Of the total 12 patients in the West Midlands with tyrosinaemia I, 10 (83%) were of non-oriental Asian ethnicity; the incidence of tyrosinaemia I was 3.7/10⁶ head of population in this group and 0.04/10⁶ in the rest of the population.

Conclusions—Asians in the West Midlands have a high incidence of tyrosinaemia I. Neonatal PKU screening using amino acid chromatography may contribute to diagnosis and early treatment.

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In 1969 the United Kingdom introduced a national neonatal screening programme for the detection of phenylketonuria (PKU; McKusick 26160). This entailed the measurement of phenylalanine concentrations in blood samples collected by skin puncture between six

and 10 days after delivery. The choice of analytical method for phenylalanine was left to individual laboratories. Methods available include the Guthrie microbiological assay¹ and one-dimensional amino acid chromatography.² The former is a specific assay for phenylalanine; the latter can also demonstrate increased concentrations of several other amino acids including tyrosine.

Tyrosinaemia type I (fumaryl acetoacetase deficiency; McKusick 276700) is an inborn error of tyrosine metabolism with an estimated worldwide incidence of 1 in 100 000 to 1 in 120 000.^{3,4} Clinical presentation may be with hepatic failure during the first year of life, a Fanconi syndrome with rickets, or acute porphyric-like episodes.^{4,5} Hepatocellular carcinoma develops usually during the second decade. The disease is characterised biochemically by increased plasma concentrations of tyrosine. Definitive diagnosis is by demonstration of increased urinary excretion of succinylacetone, and by assay of fumaryl acetoacetase activity in leucocytes, fibroblasts, or hepatocytes. Plasma concentrations of phenylalanine and/or methionine are often increased as a result of hepatic dysfunction.

Amino acid chromatography may demonstrate increased concentrations of tyrosine in plasma or whole blood in a proportion of patients with tyrosinaemia at 6 days of age, although benign transient neonatal hyper-tyrosinaemia (McKusick 276500) may give indistinguishable findings.⁶ Increases in plasma phenylalanine caused by hepatic disease may be detectable in neonates using the Guthrie assay, as has been observed in patients with galactosaemia (McKusick 23040).⁷ Although neonatal screening programmes for tyrosinaemia in the United Kingdom have not generally been regarded as cost effective, because of the relatively low incidence of the disease and the lack of an effective treatment to alter the long term outcome, the success of liver transplantation⁸⁻¹⁰ and 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3 cyclohexanedione (NTBC) treatment¹¹⁻¹³ suggest that it is appropriate to reassess this.

We therefore assessed the prevalence of tyrosinaemia in the West Midlands Regional Health Authority (RHA), and the ability to detect it using current screening programmes for phenylketonuria (PKU), based on chromatography and on the Guthrie test, using retrospective analysis of data over almost 10 years.

Methods

The West Midlands RHA, as defined before April 1994, covers the counties of the West Midlands, Worcester, and Hereford,

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Table 1 Diagnosis of tyrosinaemia following neonatal screening for PKU in the West Midlands RHA between January 1985 and March 1994

	Birmingham	Rest of RHA	Total
Known births	145 534	525 184	670 718
Screening refused	90 (0.06%)	33 (0.006%)†	123 (0.02%)
Neonates screened	145 444	525 151	670 595
Retested at 6 weeks age for increased tyrosine	447 (0.3%)	163 (0.03%)†	610 (0.1%)
Increased tyrosine at 6 weeks	6	0	6
<i>Diagnoses of persistent hypertyrosinaemia:</i>			
Before screening	0	1	1
At screening	6*	0	6*
Not detected by screening	2	4	6
Incidence of tyrosinaemia I	1 in 20 791†	1 in 105 037	1 in 55 893
95% Confidence intervals	1 in 11 943–1 in 80 208	1 in 55 974–1 in 850 738	1 in 35 696–1 in 128 726

*Includes one patient with 4-hydroxyphenylpyruvate dioxygenase deficiency (tyrosinaemia type III), and five with tyrosinaemia type I.
†P<0.001 vs whole of West Midlands RHA.

Shropshire, Staffordshire and Warwickshire. Demographic information for the RHA and for individual districts of the West Midlands County was obtained from the 1991 National Census.^{14 15} Data on birth rates were available from neonatal screening laboratory records.

For historical reasons, the neonatal screening service within the West Midlands RHA uses two different methods for the detection of PKU. Within the health authorities serving Birmingham, one-dimensional amino acid chromatography of plasma anticoagulated with heparin is used as the screening test for all specimens. In the rest of the West Midlands Region, the Guthrie microbiological method is used to detect increased phenylalanine concentrations in dried blood spots, with confirmation of abnormal results by amino acid chromatography.

All specimens received as part of the neonatal screening programme for PKU from children born between 1 January 1985 and 31 March 1994 were included in the study. The protocol for investigation was as follows: when amino acid chromatography (used either as the first line test or to confirm an abnormal result on the Guthrie test) showed an increased plasma tyrosine, a screening test for galactose-1-phosphate uridyl transferase activity was performed and (when plasma was available) alkaline phosphatase, aminotransferase, and γ -glutamyl transpeptidase activities were assayed. When these were abnormal, further investigations were performed as guided by the clinical situation. Otherwise, a repeat specimen for amino acid chromatography was requested when the child was aged 6 weeks. If tyrosine

remained increased at this time, either quantification of urinary succinylacetone excretion (before 1989) or a screening test for increased excretion was performed as part of a protocol to investigate for potential causes. Positive results were confirmed by assay of fumaryl acetoacetase activity, usually in cultured fibroblasts. Clinical and biochemical information on patients diagnosed within the West Midlands was collated by the Clinical Chemistry Department at the Children's Hospital, Birmingham, which since 1991 has provided a national service for assay of fumaryl acetoacetase activity. In most cases (in particular, when liver transplantation or treatment with NTBC were considered) patients were reviewed clinically at the same hospital, which is a supraregional centre for management of inherited metabolic and paediatric liver diseases, and one of two centres performing paediatric liver transplantation in the United Kingdom.

The incidence and prevalence of tyrosinaemia type I within subgroups were compared with that in the whole of the West Midlands by means of the binomial distribution. Other comparisons were performed using the χ^2 test. The MINITAB 8 statistical program (Minitab Inc, Pennsylvania) was used for all comparisons.

Results

Between January 1985 and March 1994, 670 718 babies were born within the West Midlands, 145 534 within Birmingham and 525 184 outside. The detection of hypertyrosinaemia both by neonatal screening and by subsequent clinical presentation is shown in table 1, and patient details provided in table 2.

Within Birmingham, amino acid chromatography showed an increased plasma tyrosine concentration in 447 neonates (0.31% of those screened). Of these, one presented with a chest infection and abnormal coagulation at 8 days of age, and was found to have tyrosinaemia type I. A further five infants (0.004%) had persistently increased plasma tyrosine at 6 weeks of age. One child (case 6) had 4-hydroxyphenylpyruvate dioxygenase deficiency (tyrosinaemia type III; McKusick 276710), and the other four had tyrosinaemia type I. One (case 5) had ascites at the time of repeat testing, while the rest showed no clinical evidence of disease at the time of diagnosis.

Table 2 Characteristics of patients with tyrosinaemia

Screening method	Case No	Detected on screening?	Age at diagnosis	Clinical features at diagnosis
AAC	1	Yes	8 Days	Pneumonia, prolonged prothrombin time
	2	Yes	6 Weeks	Asymptomatic
	3	Yes	6 Weeks	Asymptomatic
	4	Yes	7 Weeks	Asymptomatic
	5	Yes	7 Weeks	Ascites
	6	Yes	6 Weeks	Developmental delay
	7	No	12 Weeks	Hepatic failure
	8	No	35 Weeks	Rickets, hepatomegaly
Guthrie	9	No	2 Days	Hypoglycaemia
	10	No	7 Weeks	Hepatic failure
	11	No	13 Weeks	Hepatic failure
	12	No	105 Weeks	Rickets
	13	No	125 Weeks	Rickets

Case 6 had 4-hydroxyphenylpyruvate dioxygenase deficiency (tyrosinaemia type III); all other patients have tyrosinaemia type I.

Two other children born in Birmingham have since presented clinically with tyrosinaemia type I. In one (case 8), the initial screen at 10 days of age was normal. The other (case 7) showed an increased plasma tyrosine on the initial screen, but a repeat specimen collected at 3 weeks of age was reported as normal. Thus when amino acid chromatography was used as the initial screening test for PKU, the false positive detection rate for disorders of tyrosine metabolism was 0.32% at 6 days, but had fallen to 0% (diagnostic specificity 100%) by 6 weeks. The diagnostic sensitivity was 71% (95% confidence interval 38–100%) for detection of tyrosinaemia type I.

In the rest of the West Midlands RHA, demonstration of an increased whole blood phenylalanine by the Guthrie test led to the discovery of an increased whole blood tyrosine in 163 children (0.03%); none of these was subsequently found to have tyrosinaemia. Tyrosinaemia type I has been diagnosed in five (0.001%) children born in this part of the West Midlands RHA, one of whom presented with hypoglycaemia and acute renal failure aged 2 days. Whole blood phenylalanine was normal at 6–10 days of age in the other four, and amino acid chromatography was therefore not performed.

Eleven of the 13 patients with disorders of tyrosine metabolism were of non-oriental Asian descent. We therefore correlated their place of birth with the local ethnicity. The children of Caucasian descent were born in areas where less than 5% of the population in 1991 was of non-oriental Asian ancestry. The remaining 11 were born in areas where over 5% were of Asian ancestry; in Birmingham, where eight children were born, those of non-oriental Asian descent formed 14.1% of the population. The number of cases observed annually by district was 0.07/10⁶ head of population in areas with <5% non-oriental Asian descent (95% CI 0.03 to 0.17/10⁶), and 0.53/10⁶ in areas with >5% Asian descent (95% CI 0.20 to 0.86/10⁶; $P < 0.05$ *vs* whole of West Midlands). By ethnic group, the annual number of cases in the whole of the West Midlands was 3.7/10⁶ among those of non-oriental Asian descent (95% CI 1.4 to 6.1/10⁶; $P < 0.0001$ *vs* incidence in the entire population) and 0.04/10⁶ (95% CI <0.01 to 0.11/10⁶) in the rest of the population. A high proportion of the non-oriental Asian patients with tyrosinaemia type I (eight out of 10) are known to be the products of consanguineous (first cousin) marriages and the mothers of two of these eight patients are second cousins. The parents of both white patients are unrelated.

Discussion

This study has confirmed that disorders of tyrosine metabolism can be detected using the existing neonatal screening programme for PKU with an amino acid chromatographic assay. The small number of patients born outside Birmingham did not have liver disease severe enough to produce an increase in plasma phenylalanine detectable by the

Guthrie test at 6–10 days of age, although amino acid chromatography, if performed, may have shown an increased blood tyrosine. We have also demonstrated a fivefold increase in the incidence of tyrosinaemia type I within Birmingham (1 in 20 791) compared with that in the rest of the West Midlands region (1 in 105 037). This latter figure is comparable with the incidence reported worldwide.^{3,4} We may have underestimated the incidence of tyrosinaemia, as some children may have died without the diagnosis being recognised, or may have failed as yet to present clinically. The latter is unlikely, as most patients present within the first two years of life.^{3,5,16} We consider it unlikely that we are unaware of any patients with confirmed tyrosinaemia within the West Midlands region, because of the biochemical and clinical interest within this regional centre in liver disease in general, and tyrosinaemia in particular.

The increased incidence of tyrosinaemia observed within Birmingham seems to be associated with non-oriental Asian ethnicity and consanguinity. Classification of births by parental ethnicity was not available during the period of this study. To obtain some measure of the incidence of tyrosinaemia in different populations, we therefore compared the number of annual cases with the size of the appropriate ethnic population. This approach is subject to variations in family size, but suggests that tyrosinaemia type I is about 90 times more common among those of non-oriental Asian descent than among the white population. A high incidence of tyrosinaemia type I is recognised among French Canadians from the Saguenay-Lac Saint-Jean region of Quebec and also among Scandinavians,⁴ but has not to our knowledge been described before in Asians. Our results suggest that the disease may be underdescribed within the Indian subcontinent.

A high proportion of false positive results are found at 6–10 days of age when amino acid chromatography is used to screen for raised plasma tyrosine, necessitating a repeat assay several weeks later. This repeat assay has high specificity; but the delay in diagnosis permits a proportion of children (23% in this study) to develop clinical features of liver disease before diagnosis. A method of confirming the diagnosis of tyrosinaemia type I at the time of the initial screen would permit earlier diagnosis and treatment. Options include the assay of succinylacetone in urine, serum, or dried blood spots,¹⁷ immunoreactive fumaryl acetoacetase in blood spots,¹⁸ or porphobilinogen synthase activity in blood spots.¹⁹ None of these currently seems ideal. Succinylacetone production seems to depend on diet.¹⁸ Assay of immunoreactive fumaryl acetoacetase is currently in use in Quebec, where 90% of patients are homozygous for a mutation associated with absence of immunoreactive protein.²⁰ Porphobilinogen synthase activity has yet to be evaluated in a screening programme, and its use is likely to be subject to the same limitations as that of succinylacetone. A prospective comparison of these approaches is required to assess their relative merits.

Our failure to detect all patients with tyrosinaemia born in Birmingham is disappointing. One case was detected on the initial amino acid chromatography screen, but was classified as normal on repeat testing. The other patient did not have an increased plasma tyrosine at the time of the initial screen. The reason for this is unclear. Midwives collecting specimens for PKU screening are instructed to indicate that neonates have been established on milk for at least 72 hours, and a repeat sample is requested if this condition is not fulfilled. However, feeding may have been withdrawn temporarily in the infant in whom repeat testing was normal. One or two patients in whom plasma tyrosine was normal or only slightly raised at diagnosis have been reported.^{4,21} In the Quebec screening programme mean plasma tyrosine at diagnosis has fallen progressively from 854 $\mu\text{mol/l}$ in 1970–74 to 567 $\mu\text{mol/l}$ in 1990–91. The lowest reported plasma tyrosine in a neonate with tyrosinaemia diagnosed through that programme was 210 $\mu\text{mol/l}$, the threshold for further investigation being set at 200 $\mu\text{mol/l}$. A high index of suspicion and careful choice of the threshold value seem necessary to avoid false negative results, on the one hand, and a disproportionate number of false positives (requiring further investigation) on the other. We are currently assessing quantitative assays for tyrosine and succinylacetone, as use of such assays may allow more reliable detection than amino acid chromatography of patients with mild increases in plasma tyrosine.

Detection of tyrosinaemia by the protocol used in this study was a modest addition to the PKU national screening programme, and the cost of the initial tests was covered by that programme. We have not performed a detailed assessment of the costs associated with the review of neonates in whom tyrosine is raised, but the laboratory costs for this are about £300 a year. Unfortunately, we are unable to quantify the cost of nursing time involved in collecting repeat specimens. The choice of appropriate technology nationally for neonatal screening is currently under review, and this may have a significant impact on the cost of screening for tyrosinaemia. Further assessment of the place of NTBC in treatment is required before a full cost-benefit analysis can be performed.

This study confirms that tyrosinaemia type I is relatively common among Asian children in the West Midlands, and that a proportion of patients can be detected by neonatal screening using amino acid chromatography. Further work is required to increase the sensitivity of screening, and to evaluate the most appropriate

diagnostic tests which can be performed using the initial screening specimen. We suggest that neonatal screening for tyrosinaemia type I merits further assessment, particularly in areas where a high proportion of the population is of Asian ethnicity.

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