Measurement of activity of urea resistant neutrophil alkaline phosphatase as an antenatal screening test for Down's syndrome

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Abstract

Objective—To investigate the value of measuring maternal urea resistant neutrophil alkaline phosphatase activity as an antenatal screening test for Down's syndrome.

Design—Case-control study of blood samples collected at nine to 27 weeks of pregnancy.

Setting—Antenatal clinics in London and Oxford.

Patients—72 Women whose fetuses had been diagnosed by amniocentesis or chorionic villus sampling as having Down's syndrome and 156 women whose fetuses did not have the syndrome. Only singleton pregnancies were studied.

Main outcome measure—Activity of urea resistant neutrophil alkaline phosphatase measured cytochemically.

Results—The median enzyme activity in the index patients was 1.65 times the expected median for the controls at the same duration of pregnancy ($p < 0.0001$; 95% confidence interval 1.56 to 1.74). A cut off value that identified the 5% of control patients with the highest activities yielded a rate of detection of Down's syndrome of 79% (95% confidence interval 70 to 89%).

Conclusion—Activity of urea resistant neutrophil alkaline phosphatase is an effective maternal blood marker for Down's syndrome. Its use in antenatal screening could lead to a substantial improvement in the detection of this disorder. Before introducing the test into routine medical practice it will have to be automated so that it can be used on a large scale and is less subjective.

Introduction

Several substances are present at abnormally high or low concentrations in serum from pregnant women whose fetuses have Down's syndrome. For three of them— α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin—the association is strong enough for their measurement to form the basis for antenatal screening for Down's syndrome in early pregnancy,¹ whereas for others such as pregnancy specific β_1 glycoprotein, progesterone, and dehydroepiandrosterone sulphate the association is weaker.^{2,4} In a study of four pregnant women whose fetuses had been diagnosed as having Down's syndrome three of the women had raised activities of urea resistant neutrophil alkaline phosphatase; in the fourth, who did not, the pregnancy ended in an intrauterine death.⁵ This observation together with the finding that activity of urea resistant neutrophil alkaline phosphatase was, on average, raised in 33 women who in the past had had a pregnancy in which the fetus had Down's syndrome⁶ prompted us to investigate whether measurement of this activity might be a useful antenatal screening test for fetal Down's syndrome.

Patients and methods

During 1987-9 the department of environmental and preventive medicine at St Bartholomew's Hospital was

notified whenever Down's syndrome was diagnosed antenatally in any of the eight cytogenetics laboratories in the four Thames regional health authorities. The study was restricted to singleton pregnancies. With the agreement of the consultants concerned we contacted the women in whom the diagnosis had been made (the index patients) and invited them to provide a blood sample. All 72 who were invited agreed; Down's syndrome had been diagnosed after amniocentesis in 70 and after chorionic villus sampling in two. The blood sample was collected at a median of 19 weeks of pregnancy, before a therapeutic abortion; a blood film was made immediately.

Forty six women with singleton pregnancies in which the fetus did not have Down's syndrome (controls) were identified in the same way as the index patients (after amniocentesis in 45 and after chorionic villus sampling in one) after confirmation that the fetus had a normal karyotype. The blood samples were collected at a median of 21 weeks of pregnancy. This was later than in the index patients because the interval between amniocentesis (or chorionic villus sampling) and the collection of blood for measurement of the enzyme activity was longer in the controls: the cytogenetics laboratories usually reported aneuploidy straight away but reported a normal karyotype only after less severe chromosomal changes had been excluded. To obtain more controls at an earlier stage of pregnancy so that enzyme activities in the index patients could be compared with those in controls whose pregnancy was of the same duration we identified 110 women with singleton pregnancies attending their first routine antenatal clinic at the John Radcliffe Maternity Hospital, Oxford, who provided blood samples.

Blood films from the 72 index patients and 156 controls were stained for urea resistant neutrophil alkaline phosphatase in the department of haematology at St Bartholomew's Hospital. A semiquantitative technique was used to measure the activity of the enzyme.⁷ Blood films were fixed in a solution of methanol formaldehyde for one minute, washed, dried, and then stored at -20°C until required. Before being stained the films were treated with 1.5M urea in 0.1M Tris buffer (Sigma), pH 9.8, at 37°C for 15 minutes. They were then incubated with β naphthol AS-BI phosphate (Sigma) as a substrate plus fast red violet LB (Sigma) as a coupling diazodye at pH 10.0 at 37°C for 15 minutes, washed briefly in tap water, and counterstained with aqueous haematoxylin for five minutes. One hundred neutrophils on each slide were individually scored according to the intensity of the staining of the cytoplasmic granules (0=no granules, stain absent or faint; 1=few scattered granules, stain pale pink; 2=many regularly dispersed granules, stain moderately pink; 3=numerous granules throughout the whole of the cytoplasm, stain dark pink to red). The scores were summed for each slide. The scoring was done by one of two people, who did not know whether the slide was from an index patient or a control. There was a systematic absolute difference between the scorers' total scores per slide, and to allow

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for this in the analysis the average difference in score between them (55) was added to the lower scorer's results. As the scores increase with advancing pregnancy in early pregnancy they were expressed as multiples of the expected (normal) median for the control patients whose pregnancy was of the same duration. The expected medians were obtained by linear regression of the observed control median values for each week of pregnancy on the median duration of pregnancy in days, weighted by the number of women at each week. Duration of pregnancy was measured from the first day of the last menstrual period.

TABLE I—Number (percentage) of patients whose fetuses had Down's syndrome and controls with urea resistant neutrophil alkaline phosphatase activity greater than or equal to various multiples of the normal median at same duration of pregnancy

Multiples of the median	Index patients (n=72)	Controls (n=156)
≥1.65	36 (50)	2 (1)
≥1.60	41 (57)	2 (1)
≥1.55	48 (67)	2 (1)
≥1.50	49 (68)	2 (1)
≥1.45	53 (74)	6 (4)
≥1.40	57 (79)	8 (5)
≥1.35	60 (83)	13 (8)
≥1.30	64 (89)	18 (12)
≥1.25	64 (89)	30 (19)
≥1.20	66 (92)	42 (27)

Results

The figure shows the individual scores for urea resistant neutrophil alkaline phosphatase for the index patients and controls according to week of pregnancy (from nine to 27 weeks). A line representing the normal median in the controls is shown together with lines representing 0.5 and 1.5 multiples of the median, which are roughly equivalent to the 10th and 90th centiles, respectively, in the controls. The median in the index patients was 1.65 multiples of the normal median ($p < 0.0001$ compared with the controls; 95% confidence interval 1.56 to 1.74).

Table I shows the proportions of index patients and controls whose scores were greater than or equal to various multiples of the normal median. At a cut off that identified 5% of control patients as having increased enzyme activity (1.40 multiples of the median) the corresponding detection rate for Down's

syndrome was 79% (95% confidence interval 70 to 89%).

Table II shows the median and 25th and 75th centiles of urea resistant neutrophil alkaline phosphatase activity in the index and control patients according to maternal age, the indication for amniocentesis (or chorionic villus sampling), and the time between that procedure and measurement of the enzyme activity. There was no obvious association between the enzyme activity and any of these three variables.

Discussion

We confirmed the observations of Grozdea *et al* that blood urea resistant neutrophil alkaline phosphatase activity is raised in pregnant women whose fetus has Down's syndrome³; we found that the median activity was 1.65 times that in women whose fetuses did not have the syndrome. When a cut off value of 1.40 multiples of the normal median was used 79% of the index patients were identified with a rate of false positive results of 5%. At present the single most effective marker for Down's syndrome is human chorionic gonadotrophin concentration, which detects 37% of women whose fetuses have the syndrome at a cut off that yields a 5% false positive rate (derived from gaussian distributions with the parameters reported previously¹). Measurement of urea resistant neutrophil alkaline phosphatase activity is therefore a more discriminating test. To our knowledge no other single blood test can identify Down's syndrome so effectively.

We do not know why the activity of urea resistant neutrophil alkaline phosphatase is raised in pregnant women whose fetuses have Down's syndrome, although it may be related to the observation that this activity is raised in people with Down's syndrome.⁸ The enzyme activity is also raised in women who in the past have had a pregnancy in which the fetus had Down's syndrome,⁶ an observation that cannot be readily explained by a direct fetal effect unless the fetus provides a signal to the mothers' neutrophils or a neutrophil precursor that persists after the pregnancy.

Our results were not influenced to any great extent by the fact that most samples were collected after amniocentesis or chorionic villus sampling as in the controls the medians were similar in the women who did (1.05 multiples of the normal median) and did not (1.00 multiples of the normal median) have either procedure (table II). Moreover, there was no correlation between the enzyme activity and the time since amniocentesis or chorionic villus sampling.

At present screening for Down's syndrome is best done by measuring α fetoprotein, unconjugated oestriol, and human chorionic gonadotrophin concentrations; these together with maternal age can identify 61% of pregnancies in which the fetus has Down's syndrome with a false positive rate of 5%.¹ Adding measurement of urea resistant neutrophil alkaline phosphatase activity will increase the detection rate for the same false positive rate, but the test is not yet ready for routine use. More information is needed on the performance of the test earlier in pregnancy, at about 16 weeks, when the other screening tests for Down's syndrome and neural tube defects are carried out. The shape of the frequency distributions of the enzyme activity in women whose fetuses do and do not have the syndrome needs to be more precisely specified and the correlation, if any, with the other markers estimated. Also, the test needs to be automated to make it less labour intensive and less subjective. When this has been accomplished it should be a useful practical advance in antenatal screening for Down's syndrome.

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TABLE II—Median (25th and 75th centiles) urea resistant neutrophil alkaline phosphatase activity (expressed as multiples of the normal median at same duration of pregnancy) in patients whose fetuses had Down's syndrome and controls according to maternal age, time between diagnostic procedure (amniocentesis or chorionic villus sampling) and enzyme test, and reason for diagnostic procedure

	Index patients			Controls		
	No	Median	(25th, 75th centiles)	No	Median	(25th, 75th centiles)
Maternal age (years):						
≤37	15	1.68	(1.41, 2.10)	138	1.00	(0.76, 1.21)
38-39	16	1.51	(1.15, 1.79)	11	1.06	(0.65, 1.34)
≥40	41	1.73	(1.50, 1.95)	7	1.08	(1.03, 1.27)
Interval between diagnostic procedure and enzyme test (days):						
≤23	58	1.67	(1.48, 1.92)	5	0.95	(0.84, 1.40)
24-25	4	1.36	(0.91, 1.99)	6	0.99	(0.72, 1.26)
26-27	6	1.88	(1.46, 2.18)	6	0.93	(0.53, 1.24)
≥28	4	1.49	(1.41, 1.75)	29	1.06	(0.66, 1.25)
No diagnostic procedure				110	1.00	(0.76, 1.21)
Indication for diagnostic procedure:						
Advanced maternal age	64	1.67	(1.47, 2.00)	18	1.13	(0.83, 1.27)
Low α fetoprotein	5	1.38	(1.17, 1.75)	26	1.00	(0.58, 1.08)
Other	3	1.73	(1.44, 1.76)	2	1.07	
No diagnostic procedure				110	1.00	(0.76, 1.21)
Total	72	1.65	(1.42, 1.93)	156	1.02	(0.76, 1.21)

Urea resistant neutrophil alkaline phosphatase activity according to duration of pregnancy in 72 women whose fetuses had Down's syndrome (●) and 156 controls (○)

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Accuracy of clinical diagnosis of Alzheimer's disease

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There is no specific antemortem marker for Alzheimer's disease, and the diagnosis may be uncertain even after examination of cerebral tissue.¹ In 1988 Homer *et al* emphasised the problems of correctly predicting the pathological findings in various types of dementia and implied that Alzheimer's disease was often not confirmed at necropsy.² The aim of our study was to assess prospectively the ability of current rigorous clinical criteria to diagnose Alzheimer's disease successfully during life.

Patients, methods, and results

We studied elderly psychiatric patients living in the area covered by Camberwell Health Authority, London. Altogether 180 were enrolled in the study, of whom 86 had died by three years. Necropsy was performed on 54 patients, and we report here on the first 50.

The clinical criteria used were those of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.³ Probable Alzheimer's disease was diagnosed in patients with a history of a slowly progressive dementia without concomitant physical illness. Possible Alzheimer's disease was diagnosed in patients with atypical features of the disease or physical illness. Definite Alzheimer's disease was diagnosed only when the disease was confirmed by histopathological examination—that is, by the finding of widespread neocortical and hippocampal disease with plaques or neurofibrillary tangles, or both. Diffuse Lewy body disease was diagnosed when Lewy bodies were found not only in the substantia nigra and

locus coeruleus but also in the cortex, particularly in the parahippocampal gyrus. Vascular damage was considered to be important when multiple, widespread macroscopic or microscopic lesions were identified.

The average age of the 50 patients studied was 82.3 years (range 70-99). The table shows the numbers of patients predicted by the clinical criteria to have probable and possible Alzheimer's disease and the neuropathological diagnoses. The criteria for predicting probable Alzheimer's disease had a sensitivity of 88% (28/32), and those for predicting possible Alzheimer's disease had a sensitivity of 78% (14/18). We could not assess specificity as we did not include in the study patients who did not satisfy the clinical criteria for the disease. The table also shows the ability of the criteria to exclude patients with vascular disease. Two patients had evidence of only vascular disease; in both cases the criteria had predicted possible Alzheimer's disease.

Comment

The main finding of this study was that Alzheimer's disease can be diagnosed accurately during life by strictly applying the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association without the need for extensive investigation and resources. The rate of agreement between clinical and histological diagnoses of the disease varies from 50%² to over 95%,⁴ but studies in which agreement has been 90% or above have all used the criteria that we used here. This is the first study to attempt to validate the criteria for possible Alzheimer's disease; it shows that the disease may be diagnosed in patients with other physical illness (such as diabetes and hypertension).

Cortical Lewy body disease was found in six patients (12%), a prevalence similar to that found by Perry *et al*,⁵ which suggests that this disease is the second commonest neurodegenerative dementia in old age.

The ability of the clinical criteria that we used to diagnose Alzheimer's disease in 88% of patients confirms their validity. Such successful clinical prediction of disease is unusual in any branch of medicine.

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Relation between diagnosis of Alzheimer's disease (probable or possible) based on clinical criteria and neuropathological diagnosis, and ability of clinical criteria to predict neuropathological findings of Alzheimer's disease and exclude vascular disease

	Diagnosis of Alzheimer's disease from clinical criteria		Total (n=50)
	Probable (n=32)	Possible (n=18)	
Neuropathological diagnosis:			
Alzheimer's disease alone	24	10	34
Alzheimer's disease and vascular disease	2	3	5
Alzheimer's disease and cortical Lewy body disease	2	1	3
Vascular disease alone		2	2
Cortical Lewy body disease alone	3		3
No diagnosis	1	2	3
Ability of clinical criteria to predict pathological findings of Alzheimer's disease:			
Findings present	28	14	42
Findings absent	4	4	8
Ability of clinical criteria to exclude vascular disease:			
Vascular disease absent	30	13	43
Vascular disease present	2	5	7