PAPERS AND ORIGINALS

Detection and measurement of fetomaternal haemorrhage: serum alpha-fetoprotein and the Kleihauer technique

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Summary

A raised maternal serum alpha-fetoprotein concentration was taken as an indicator of fetomaternal haemorrhage due to amniocentesis and was used to calculate the volume of the fetal bleed. The alpha-fetoprotein concentration seemed to be a more sensitive and reliable indicator than the established Kleihauer technique and may have further applications in antenatal testing.

Introduction

It has been appreciated for some time that samples for the assay of maternal serum alpha-fetoprotein (α-FP) in the antenatal diagnosis of neural tract disorder should be taken before amniocentesis.[1] Spuriously high results have been obtained in this laboratory, and in others with postamniocentesis samples.[2] Fetomaternal haemorrhage (FMH) is assumed to cause these spurious results. We therefore measured α-FP levels in samples taken before and after amniocentesis to determine any FMH and compared the results with those obtained with the Kleihauer technique to assess which method detected FMH more accurately.

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Patients and methods

Blood samples were collected immediately before and 15 minutes after amniocentesis in 113 patients; their gestational ages ranged from 14 weeks to 40 weeks. Amniocentesis was performed for antenatal diagnosis (58 cases), fetal maturity assessment (53 cases), and rhesus isoimmunisation (2 cases).

α-FP was estimated by a single antibody radioimmunoassay technique based on the method of Leek and Chard.[3] Standard α-FP and ^{125}I-labelled α-FP preparations were obtained from Abbott GmbH, the standards being based on the α-FP preparations of Dr Shinsho Nishi. Antisera to human α-FP were obtained from Dakopatts, Copenhagen. Standardisation was further checked by reference to the British Standard α-FP preparation 72/227 rated at 56 mg/l.[4]

The criterion of a significant increase in serum α-FP between pre- and post-amniocentesis samples was established on the basis of the within-assay coefficient of variation (7%). We concluded that a haemorrhage had occurred if the α-FP level in the post-amniocentesis sample was 40% or more above that in the pre-amniocentesis sample. In one case in which FMH occurred serum samples taken 15, 30, and 60 minutes after amniocentesis showed complete mixing of α-FP in the maternal circulation within 15 minutes; this finding accords with other published results.[5] In calculating the FMH volume we allowed for median values of fetal serum α-FP, maternal plasma volume, and fetal packed cell volume at the respective gestations.

The Kleihauer technique for counting fetal erythrocytes in maternal blood was performed according to Harwood's modification of the method.[6] A significant haemorrhage was considered to have occurred if the difference in the fetal red cell count per 50 low power fields (LPF) was 5 or more, and an increase of 5 cells/50 LPF was taken as equivalent to a fetal bleed of 0·25 ml.

Results

Of the 113 cases studied, 96 (85%) showed no indication of FMH by either the α-FP concentration or the Kleihauer method. Table I shows the 17 cases (15%) in which there was evidence of FMH: in five this was shown by both the α-FP and the Kleihauer methods, a further 10 showed significant changes in the α-FP concentration only,

and two showed abnormal Kleihauer results with stable α-FP levels. Table II shows the calculated volume of FMH by both techniques.

The incidence of detectable FMH after amniocentesis in this series was 13% when assessed by the α-FP concentration and 6% when assessed by the Kleihauer method.

TABLE I—*Details of the 17 cases of fetomaternal haemorrhage: results of α-FP and Kleihauer methods in samples taken before and after amniocentesis*

Case No	α-FP (μg/l)		Kleihauer (FC/50 LPF)	
	Before	After	Before	After
Indicated by both α-FP and Kleihauer methods				
6	77	1051	0	42
9	115	202	0	440
42	22	248	0	13
58	33	1609	0	78
101	92	539	0	1663
Indicated by α-FP method alone				
7	32	76	0	2
13	26	61	0	1
15	33	1459	31	24
28	31	57	0	0
40	14	83	0	0
62	10	21	0	0
72	32	85	0	0
83	33	73	0	0
85	104	144	7	9
86	42	97	0	0
Indicated by Kleihauer method alone				
23	40	50	1	22
81	<10	<10	0	190

TABLE II—*Size of fetal haemorrhages as estimated by serum α-FP and Kleihauer methods*

Case No	Gestation (weeks)	Serum α-FP concentration			Kleihauer method
		Total α-FP increase in maternal circulation (ng)	Possible contribution by 1 ml liquor α-FP (ng)	Fetal bleed (ml)	Fetal bleed (ml)
6	16	2922	4·7	2·04	2·1
7	15	131	6·7	0·08	NS
9	37	326		3·14	22
13	14	103	19	0·06	NS
15	16	4278	6·3	2·99	0
23	40	NS	0·07	0	1·05
28	14	77	7·5	0·05	0
40	13	202	8·2	0·11	0
42	16	678	7·6	0·47	2·65
58	15	4696	5·4	2·89	3·9
62	11	32	9	0·03	0
72	16	159	4·3	0·11	0
81	39	NS	F	0	9·5
83	37	150	0·02	1·44	0
85	15	122	F	0·08	NS
86	17	168	4·6	0·13	0
101	38	1654	F	19·08	83

NS = Not significant.
F = Failed amniocentesis.

Discussion

Increased serum α-FP levels due to amniocentesis seem to be a sensitive indicator of FMH, showing fetal haemorrhages as small as 30 μl whole blood. These calculations are only approximate since mean values have to be used throughout the calculations. The finding of such small haemorrhages may be relevant to rhesus sensitisation, since a previous primary response has been indicated in subsequent pregnancies in a few cases in which no fetal cells were detected by the Kleihauer technique in the first pregnancy.[7]

The use of an increase in maternal serum α-FP concentrations for detecting FMH has certain advantages over the Kleihauer technique. Complete mixing of the serum protein is assured in all cases and is not influenced by agglutination or aggregation of fetal cells. Aggregation of fetal cells may have distorted the Kleihauer results in one case, in which the calculated FMH would have represented a 25% fetal haemorrhage, whereas the fetal haemoglobin at delivery two days after amniocentesis was normal. α-FP concentrations seem to give a more reliable estimate of the volume of fetal blood. The volumes calculated from the two techniques agreed in only two out of 14 cases, but in a further three (cases 7, 13, and 85) the volume calculated from the α-FP increase (0·08, 0·06, and 0·08 ml respectively) would agree closely with the increase of 2, 1, and 2 cells/50 LPF respectively —results regarded as non-significant by the Kleihauer technique. The accuracy of the calculations of volume of FMH can only improve as further data on the variables concerned are obtained. The stability of α-FP under various storage conditions represents a further advantage over the Kleihauer technique, which is influenced by fetomaternal ABO incompatibility and the time interval between sampling and testing and may be completely invalidated by clotting of the sample.

Further inconsistencies were apparent with the Kleihauer test in this study. In case 15 fetal cells were present both before and after amniocentesis but showed an apparent fall with the Kleihauer method, while α-FP concentrations indicated a haemorrhage of 3 ml. Six other cases showed persistent negative results with the Kleihauer method despite considerable changes in the α-FP levels.

In case 13 the detection of FMH indicated a possible cause for a raised α-FP level in a clear amniotic fluid of 56 mg/l at 14 weeks (mean +2 log SD: 42 mg/l). A further sample two weeks later showed a concentration of 35 mg/l (mean +2 log SD: 36 mg/l). The pregnancy resulted in the delivery of a normal infant. Analysis of the first sample for haemoglobin F[8] might have clarified this finding.

An increase in maternal serum α-FP concentration after amniocentesis is positive evidence of admixture of fetal plasma. The presence of maternal antibodies to α-FP would theoretically invalidate the technique, but the existence of such antibodies has not been proved. Two cases in the total series failed to show significant changes in α-FP despite considerable differences on the Kleihauer test. Case 81 was unusual in that the maternal serum α-FP concentration was <10 μg/l and FMH was indicated by the Kleihauer method after two failed amniocenteses, one of which produced only urine. These sera may merit studies for antibody to α-FP. Case 23 showed a 25% increase in α-FP concentrations after amniocentesis but strict adherence to the described criteria would not allow this to be classified as indicative of FMH. The discriminant limit of 40% was selected to allow for some fluctuation in the within-assay coefficient of variation around the mean of 7% and so minimise the potential overdiagnosis of FMH. The incidence of FMH detected by the Kleihauer technique in this series was 6%, which generally agrees with that obtained in another series.[9] But the sensitivity of the increase in serum α-FP concentrations after amniocentesis gave an incidence of FMH of 13%.

The use of the α-FP technique was restricted in this series to one antenatal procedure, but further applications may be found whenever analyses can be performed on samples taken before and after a manipulative procedure. The disadvantages of the α-FP techniques are the length of time of assay and the more complex calculation of the volume of FMH.

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Is cowpox misnamed? A review of 10 human cases

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Summary

Twelve separate outbreaks of confirmed cowpox, 10 involving humans, were reviewed. Six of the patients, including three children, had severe infections and five were admitted to hospital. In three outbreaks both people and cows were affected but it was not known how the infections entered the herds. In seven outbreaks no direct contact with cattle was established and clinical and serological examination failed to show evidence of cowpox in the bovine population.

Comparison of these data with information about infections known to be enzootic in cattle leads to the suggestion that cows are not the natural reservoir of cowpox. This should be remembered when diagnosis is considered. The role of small wild animals as hosts and vectors of "cowpox" should be investigated.

Introduction

The success of the World Health Organisation's Smallpox Eradication Campaign has aroused interest in the epidemiology of those poxviruses, immunologically related to smallpox virus, that infect man. Accidental infection with vaccinia virus should reduce as vaccination is discontinued. Of the other virus infections concerned only cowpox is indigenous to Britain and likely to be a public health hazard.

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Cowpox virus infection is reported in Britain only when human cases occur or when many cattle are affected. Human cowpox has usually been regarded as an occupational disease of dairy farmworkers.[1-3] A recent note,[4] however, discusses three separate cases in man in which contact with cattle was not established. Perhaps because cowpox is relatively unimportant no studies have been made on the way in which the virus is maintained and transmitted. Some suggest that cowpox is enzootic in cattle and that it is maintained by trivial infection.[1 3] Others consider cowpox to be uncommon or rare but do not suggest how the virus is maintained.[5-7]

All the above workers assume that the cow is the natural host and reservoir of cowpox virus, although the possibility that some unknown wild mammal or bird may be the reservoir has been raised.[8 9] Cowpox virus is not particularly resistant and would not survive for long in the soil. Several biotypes of cowpox virus are in circulation,[10 11] and enzootic infection in some species is necessary to ensure the survival of these biotypes.

This paper reviews 12 separate cases of confirmed cowpox infection occurring in 1965-76, 10 of them in man, in an attempt to provide information on the natural history of the disease. The biological properties of some of the isolates have already been described;[10 11] the remainder will be described elsewhere.

Cowpox is not notifiable and farmers are not obliged to allow examination of their animals. Such information as is available has been obtained through the willing co-operation of farmers and patients and the medical and veterinary workers listed at the end of this paper.

Outbreaks

Some information on the incidents is listed in table I. There was no connection between them, and they occurred in different places at different times. For convenience each incident is referred to as

TABLE I—*Some details of the 12 outbreaks reviewed*

Outbreak			Cows infected	Human cases				
No	Place	Year		Farm worker	Age*	Lesions	Days in hospital	Vaccinated
1	Tyson (N Wales)	1965	+	–	—	—	—	—
2	"188" (Somerset)	1968	+	–	—	—	—	—
3	Dorchester	1969	+	+	A	Hand	—	—
4	Winchester	1969	–	–	A	Hand	—	?
5	Middlesbrough	1971	–	–	8	Chin	24	—
6	Exeter	1971	+	+	A	Hand	—	—
7	Burnley	1974	–	–	14	Hand, chin	21	—
8	Penrith	1974	–	–	A	Hand	7	†
9	Scarborough	1975	–	–	6	Face	17	—
10	Lincoln	1975	–	–	17	Hand	8	—
11	Bristol	1976	–	–	17	Face	—	—
12	Taunton	1976	+	+	A	Hand	—	—

*A = Adult. Figures give age in years.
†Vaccinated in infancy.