

Alphafetoprotein in midtrimester Down's syndrome fetal serum

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Abstract

Serum alphafetoprotein was estimated in fetuses with and without Down's syndrome or with other chromosome abnormalities from the 17th to the 28th week of pregnancy. In normal fetuses, the AFP level declines steadily during this period. Before 20 weeks, there was no difference in the serum AFP levels of the three groups of fetuses. After 20 weeks, the serum AFP level in cases of Down's syndrome declined more rapidly than normal. This was not observed in fetuses with other chromosome abnormalities. This suggests that low maternal serum alphafetoprotein levels used for prenatal screening for Down's syndrome in the early second trimester cannot be explained by low levels in the Down's fetuses themselves.

It is now well established that midtrimester maternal serum alphafetoprotein (MSAFP) levels tend to be lower than normal when the fetus has Down's syndrome.¹⁻⁶ Some workers have found that the amniotic fluid alphafetoprotein (AFAFP) levels are below the normal median in such cases too,^{2 5-8} while others have found them to be normally distributed.^{1 4} The mechanism for these low AFP levels in the maternal serum, and possibly the amniotic fluid, is not yet known, but one suggestion has been that Down's syndrome fetuses produce less AFP than normal fetuses.⁹ My previous study¹⁰ suggested that this is not so; consequently, I have examined this point further by determining the serum AFP levels in midtrimester fetuses with and without Down's syndrome.

Materials and methods

Fetal blood samples were obtained from two sources and the results were analysed separately. In one case,

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fresh fetal blood was obtained by percutaneous umbilical cord sampling (cordocentesis) from the 18th to 28th week of pregnancy for diagnostic fetal karyotyping. In the other, postmortem blood samples were obtained by cardiac puncture from fetuses received for postmortem examination after prostaglandin termination of pregnancy, or after a midtrimester spontaneous abortion, between 17 and 26 weeks of gestation.

After either means of collection, the blood samples were spun at 6000 rpm for 10 minutes. The serum was diluted 1 in 200, 1 in 100, or 1 in 50 with distilled water according to gestational age, and the AFP level was quantified by one dimensional crossed antigen-antibody electrophoresis (the rocket technique).¹¹

Cordocentesis samples from fetuses without Down's syndrome were divided into two groups and their results were also analysed separately. Group 1 (41 samples) comprised fetuses which were completely normal, both chromosomally and developmentally. These were derived from patients undergoing the procedure for raised maternal age who presented too late for amniocentesis, or where the amniotic cell culture had failed, or where the fetus was at risk for a blood disorder. Group 2 (49 samples) consisted of fetuses who were chromosomally normal but who had a developmental defect, such as diaphragmatic hernia, hydrocephalus, congenital heart defect, or exomphalos, or there was an obstructive uropathy or Rhesus incompatibility. Samples were also received from fetuses with renal agenesis, ascites, hydrops, and intrauterine growth retardation, but they were excluded from the study.

All postmortem specimens of fetuses with Down's syndrome were derived from termination of pregnancy after prenatal diagnosis. Seven fetuses without Down's syndrome also came from terminations: five had a neural tube defect and two were normal. Serum AFP results from these were pooled with those obtained from 16 spontaneous abortions of an apparently normal fetus (group 3). Only those abortuses that were fresh, showing no sign of early maceration, were selected for study, in an attempt to obtain a comparable group to those from terminations, where the fetuses were presumably alive up to the time of the induction. Unfortunately, because of departmental policy not to perform chromosome

studies routinely on neural tube defect fetuses that are otherwise normal, or on phenotypically normal spontaneously aborted midtrimester fetuses, it was possible to confirm a normal karyotype on only six of these fetuses.

The length of gestation was calculated from the first day of the last menstrual period and correlated with the estimate of fetal age from ultrasound scan. Those in which there was no major discrepancy between the two were used.

The Mann-Whitney test was used to compare results from affected and unaffected pregnancies, directly for 19 and 20 weeks' gestation, and modified as described below for 21 to 28 weeks.

Results

Fetal serum AFP values were obtained for each week of pregnancy from 17 to 28 weeks. Results are presented from three different groups of fetuses without Down's syndrome: on fresh blood from completely normal fetuses (group 1), fresh samples from chromosomally normal but developmentally abnormal fetuses (group 2), and postmortem samples (group 3). All three groups of fetuses without Down's syndrome showed similar results (fig 1), although at some time points there were few samples. Thus,

despite differences in the type of fetus without Down's syndrome, and in the way the fetal blood samples were acquired, consistent serum AFP results for chromosomally normal fetuses were obtained. Similarly, at 19 to 20 weeks when there were both fresh and postmortem samples of Down's syndrome cases analysed, the results were comparable.

In normal fetuses the serum AFP level declined steadily and gradually over the observation period from a mean value of 2800 µg/ml at 17 weeks and 2202 µg/ml at 18 weeks to 780 µg/ml at 28 weeks.

There are AFP values on 21 fetuses with Down's syndrome (fig 1). At 19 and 20 weeks, the serum AFP levels of Down's syndrome fetuses were evenly distributed within the normal range and there was no significant difference between the two groups ($p=0.60$ for 19 weeks, $p=0.56$ for 20 weeks). By contrast, at 21 and 22 weeks, the AFP levels of all five cases of Down's syndrome fell in the lower half of the normal ranges, and at 26 weeks and 27 weeks the Down's AFP levels were below the values for normal fetuses. The numbers for each week are small, and so the results were analysed statistically by ranking (r) the values of the n samples for each fortnight (21-22, 23-24, 25-26, and 27-28 weeks), computing a score (s) for each sample ($s=[r-0.5]/n$) to give a uniform distribution for each epoch. The four sets of score

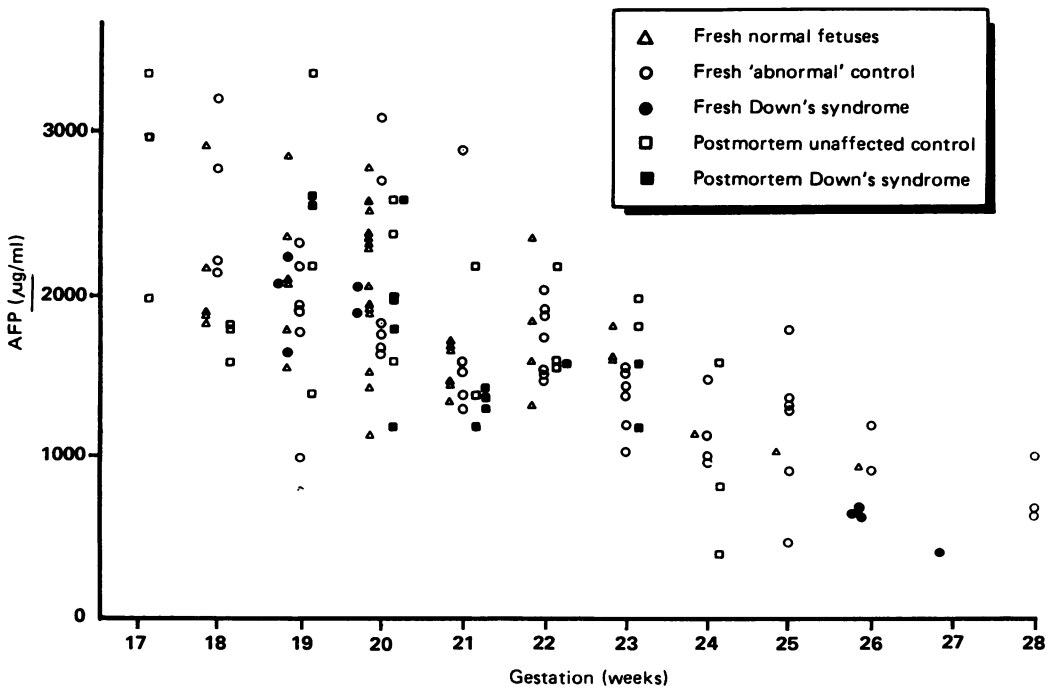


Figure 1 Serum alphafetoprotein levels in the second trimester of pregnancy in fetuses with and without Down's syndrome.

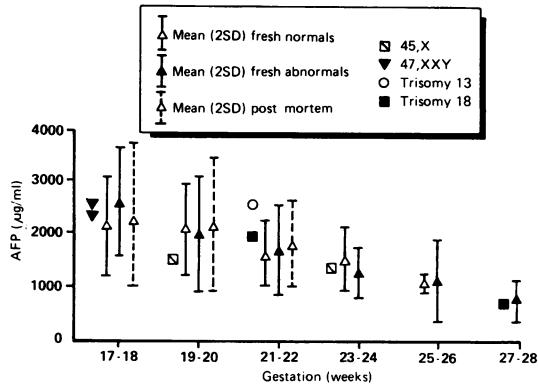


Figure 2 Serum alpha-fetoprotein levels in the second trimester of pregnancy in chromosomally normal fetuses and in those with chromosome abnormalities other than Down's syndrome.

data were then merged and a Mann-Whitney test performed on these scores, comparing the Down's syndrome results with those of the unaffected. The difference observed between the two groups was highly significant ($p=0.0033$).

Serum AFP values were also obtained for seven fetuses with chromosome abnormalities other than Down's syndrome, four of which were after 20 weeks; these are shown in fig 2. They are a disparate group (47,XXY two cases; 45,X two cases; trisomy 18 two cases; trisomy 13 one case) and few in number, but show that after 20 weeks, unlike Down's syndrome, their AFP values were similar to normal.

Discussion

Thus, before 20 weeks, the serum AFP level of Down's syndrome fetuses is the same as that of chromosomally normal fetuses. However, after 20 weeks, the AFP level of Down's syndrome fetuses shows a more rapid decline than normal and, further, this decline becomes more marked as pregnancy progresses. It is possible that fetuses with chromosome abnormalities other than Down's syndrome do not have a lower than normal serum AFP level after 20 weeks. Scioscia *et al*¹² in a series of 11 Down's fetuses, have also observed normal serum AFP levels before 20 weeks and lower levels afterwards.

These findings suggest that before 20 weeks, the production of AFP is not lower in cases of Down's syndrome than in chromosomally normal fetuses. Thus, another explanation must be sought for the fact that maternal serum AFP levels before 20 weeks are lower than normal in Down's syndrome pregnancies. After 20 weeks, however, AFP production does diminish more rapidly than normal in Down's fetuses. The observation that the decline becomes more marked as pregnancy progresses after 20 weeks, and

that at 26 and 27 weeks the AFP serum level in a Down's fetus is below that of normal fetuses, would fit with the findings of Cuckle and Wald¹³ that at birth the serum AFP level of Down's babies is 0.45 multiples of the normal median.

The contrast between findings in the early mid-trimester and at birth in Down's syndrome is seen not only with fetal serum AFP values but also in connection with body weight. Down's babies have a lower than normal birth weight when corrected for gestational age,¹⁴ while Down's fetuses terminated around 20 weeks after prenatal diagnosis have a normal body weight and growth pattern.¹⁵

It is believed that MSAFP is derived from the fetal unit, and a major route is through the membranes from the amniotic fluid. Although in neural tube defects there is only a relatively weak correlation between high AFAFP levels and the corresponding MSAFP levels,¹⁶ presumably, in the case of Down's syndrome, the low maternal serum levels do reflect the low amniotic fluid levels.

If the normal fetal serum AFP levels I have found in Down's syndrome fetuses in the early second trimester truly reflect normal liver AFP production, then an explanation for the aberrant AFP levels now perhaps has to be found at the interface of the fetus and the amniotic fluid. In the midtrimester, amniotic fluid is thought to derive its AFP mainly through fetal micturition. The possibility must now exist that excretion of AFP through the kidney is retarded in Down's syndrome fetuses in the midtrimester.

I thank Miss D Garrett, Miss B Merritt, and Mr A Webb for technical help, the cytogeneticists of the South East Thames Regional Genetics Centre for chromosome results, and the Spastics Society for financial support.

- 1 Merkatz IR, Nitowsky HM, Macri J, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. *Am J Obstet Gynecol* 1984;148:886-94.
- 2 Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. *Lancet* 1984;ii:926-9.
- 3 Seller MJ. Prenatal screening for Down syndrome. *Lancet* 1984;ii:1359.
- 4 Guibaud S, Bonnet-Capela M, Germain D, Dumont M, Thoulton JM, Berland M. Prenatal screening for Down syndrome. *Lancet* 1984;ii:1359-60.
- 5 Tabor A, Norgaard-Pedersen B, Jacobsen JC. Low maternal serum AFP and Down syndrome. *Lancet* 1984;ii:161.
- 6 Fuhrmann W, Wendt P, Weitzel HK. Maternal serum AFP as screening test for Down syndrome. *Lancet* 1984;ii:413.
- 7 Voigtlander T, Vogel F. Low alpha-fetoprotein and serum albumin levels in Morbus Down may point to a common regulatory mechanism. *Hum Genet* 1985;71:276-7.
- 8 Nelson MM, Peterson EM. Prospective screening for Down syndrome using maternal serum AFP. *Lancet* 1985;ii:1281.
- 9 Leader. Low maternal serum alpha-fetoprotein and Down syndrome. *Lancet* 1985;ii:259-60.
- 10 Seller MJ, Adinolfi M. Levels of albumin, alpha-fetoprotein, and IgG in human fetal cerebrospinal fluid. *Arch Dis Child* 1975;50:484-5.
- 11 Laurell CB. Quantitative estimation of proteins by electrophoresis

- in agarose gel containing antibodies. *Anal Biochem* 1966;15:45-52.
- 12 Scioscia A, Blakemore K, Robinson M, *et al.* Midtrimester fetal serum AFP levels in normal and Down syndrome fetuses. *Am J Hum Genet* 1988;43 (suppl 3p):250A.
 - 13 Cuckle HS, Wald NJ. Cord serum alpha-fetoprotein and Down's syndrome. *Br J Obstet Gynaecol* 1986;93:408-10.
 - 14 Chen ATL, Chan YK, Falek A. The effects of chromosome abnormalities on birth weight in man. II. Autosomal defects. *Hum Hered* 1972;22:209-24.
 - 15 Librach CL, Hogdall CK, Doran TA. Weights of fetuses with autosomal trisomies at termination of pregnancy: an investigation of the etiological factors of low serum alphafetoprotein values. *Am J Obstet Gynecol* 1988;158:290-3.
 - 16 Second report of the UK Collaborative Study on Alphafetoprotein in Relation to Neural Tube Defects. Amniotic fluid alpha-fetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. *Lancet* 1979;ii:651-62.