LOCAL NORMS OF MATERNAL SERUM ALPHA-FETOPROTEIN VALUES IN EARLY SECOND TRIMESTER: A PRELUDE TO A DOWN'S SYNDROME SCREENING PROGRAMME INCORPORATING MATERNAL SERUM ALPHA-FETOPROTEIN VALUES

F M Lai, S H Yeo, S L Yo, I K Tan

ABSTRACT

Maternal alpha-fetoprotein in (MSAFP) has recently been proposed for screening of Down's syndrome. This is based on the association of a lowered MSAFP level at a given gestational age in Down's syndrome (DS) pregnancies.

Most conventional 'screening' programmes in DS are based on the maternal age alone, selecting older mothers, who are at greater risk of having a DS infant born, for antenatal karyotyping. However, as 75% of DS infants are born to mothers less than 35 years old, a combination of blood tests including MSAFP and maternal age as independent risk factors would screen the younger mothers at a good cost-benefit ratio in selecting the 'at-risk' mothers for karyotyping.

In this study, the gestational age was calculated from the first day of the last menstrual period (LMP) as well as from ultrasound cephalometry.

The local MSAFP levels follow the same trend at a slightly higher level as those based on clinical evaluation of the Abbot AFP-EIA Monoclonal kits.

Keywords: Alpha-fetoprotein, Down's syndrome, screening, ultrasound.

SINGAPORE MED J 1991; Vol 32: 134-137

INTRODUCTION

Down's syndrome (DS) is the most common congenital cause of severe mental retardation, with an incidence at birth of about 1.3 per 1000.

The current method of antenatal 'screening' is to select women for a diagnostic amniocentesis on the basis of advanced maternal age [≥ 35 years on her estimated date of delivery (EDD)]. For women aged 35 or older, the overall likelihood of a birth of a child with DS is estimated to be one in 67 (Hook EB as quoted by 1). Ferguson-Smith & Yates in the European collaborative on 52,965 amniocenteses found 613 DS in mothers above the age of 35 ie. one in 87[2]. Age is however a poor basis for screening since as many as three quarters of Down syndrome births are acknowledged to occur in women under the age of 35.

The chance to improve the rate of detection without having to expose many more women to amniocentesis began with the observation that a low serum concentration of alpha-fetoprotein in the mother was associated with Down's syndrome in the foetus[1,3,4]. It was then shown that the serum concentration of AFP could be used independently to identify pregnancies at risk[5].

Subsequently, it was shown that better definition of a high risk group could be obtained by combining risks derived from the serum concentration of AFP in the mother and maternal age[6-8].

A study of over 18,000 pregnancies in the U.K. Collaborative Study has established multiples of the median (MoM) as the preferred way to express AFP results[9]. The median AFP value for each gestational week is first determined; then individual AFP levels are reported as a multiple of this value. This method of expression facilitates comparison of AFP test results across gestational weeks and between laboratories.

MSAFP levels normally rise during pregnancy from a normal, non-pregnant level of 0-20 ng/ml to a mean level of about 250ng/ml at 32 weeks gestation[10]. MSAFP testing is optimally done between 14 and 20 gestational weeks.

METHODS

A maternal serum alpha-fetoprotein collection programme was started at the Department of Obstetrics and Gynaecology, Singapore General Hospital (SGH) in May 1988.

In normal healthy mothers with singleton pregnancies who had an early first trimester antenatal booking, an appointment was given for maternal serum alpha-fetoprotein (MSAFP) to be taken between 14-20 weeks gestation (by LMP). These mothers were also given a routine ultrasound appointment at between 16 to 20 weeks gestation (by LMP). Data was collected at the time of venepuncture, at the time of ultrasound, at delivery and postnatally.

As it has been demonstrated that increased levels of AFP may occur in maternal serum following amniocentesis, the collection of maternal serum specimens was done prior to the amniocentesis for all cases in which amniocentesis was done.

All specimens of MSAFP collected were sent to the Biochemistry division of the Pathology Department of SGH

Department of Obstetrics & Gynaecology
Singapore General Hospital
Outram Road
Singapore 0316

F M Lai, MBBS
Resident

S H Yeo, AM, MBBS, MRCOG
Consultant

Department of Pathology
Singapore General Hospital

S L Yo, BSc, MSc, PhD
Scientific Officer

I K Tan, PhD, MCP, FRSC, FRCPath
Head of Clinical Biochemistry

Correspondence to: Dr S H Yeo

on the same day for analysis. Serum AFP concentration was measured by enzyme-immunoassay (monoclonal) using ABBOTT AFP-EIA Monoclonal kits.

Over the period of 2 years the between-run reproducibility of AFP (Abbot-EIA) performed with the Abbot Quantum for the 2 controls were as follows:

	Control I	Control II
target value	18ng/ml	111ng/ml
N	20	20
Mean	18.1	112.3
SD	1.3727	8.5055
% CV	7.5837	7.5706

Pregnancies resulting in the termination or birth of a foetus with Down's syndrome or other chromosomal abnormalities, from 2 Jan 1988 to 31 Mar 1990, were identified from the following sources:

(i) a record of all abnormal karyotyping results from amniocentesis and foetal blood sampling
(ii) the record for abnormal infants kept by the neonatology unit
(iii) a record of all mid-trimester termination of pregnancy for an abnormal foetus.

Alpha-fetoprotein values have been assigned on the basis of completed gestational weeks. For example, a specimen obtained on gestational week 18 day 6 is assigned to week 18.

As the last normal menstrual period (LMP) is prone to errors, a cut-off date of two weeks prior to the expected delivery date (EDD) on 31 Mar 1990. 17 Mar 1990 was taken as the criteria for entry into data analysis. Using this, a total of 362 samples of MSAFP were obtained. The data was entered into a computerized database and was checked against the specimen bottle forms and ultrasound records and in some cases, the old casenotes.

After excluding one sample with missing AFP result, 12 samples with LMPs which were unknown or missing and 3 samples associated with foetuses/neonates having neural tube defects or abnormal chromosomes on karyotyping, a sample size of 346 unaffected singleton pregnancies were obtained. Of these, 299 had an obstetrics ultrasound done between the LMP-based gestational age of 14 to 27.9 weeks. As the accuracy of dating by ultrasound becomes less accurate after the second trimester, only singleton pregnancies with an ultrasound done before 28 weeks amenorrhoea were selected for analysis. Out of the 299 pregnancies with an ultrasound dating, 286 satisfied the criteria of 14-20 weeks by LMP and 248 satisfied the criteria of 14-20 weeks by ultrasound cephalometry.

RESULTS

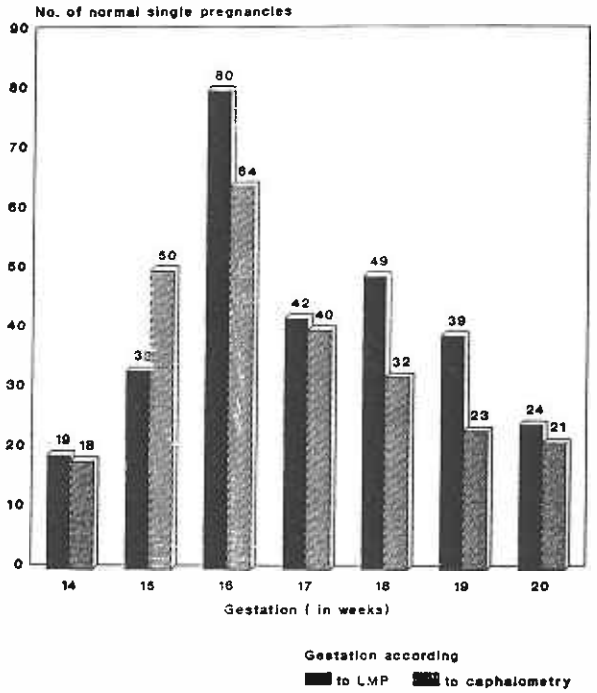
Fig 1 shows the frequency distribution of the 286 and 248 normal singleton pregnancies for which MSAFP was obtained for gestational age of 14 to 20 weeks based on the LMP and on ultrasound cephalometry respectively.

Fig 2 shows the scatter of MSAFP values from which the median for each gestational age is derived. Fig 3 shows the local median MSAFP values (in ng/ml) plotted against the gestational age (estimated by LMP and Ultrasound).

As seen in Fig 2, there are a three MSAFP values which fall far outside the range in the 19 and 20 week gestation.

The high MSAFP value of 256.7 ng/ml belonged to a patient who had active Systemic Lupus Erythematosus with nephrotic syndrome and acute renal failure. Although amniocentesis revealed 46XY karyotype, a therapeutic termination of

Fig 1 - Frequency Distribution of Number of Pregnancies by Gestational Age



pregnancy was done on medical grounds.

The patient with MSAFP of 392.0 ng/ml was a gestational diabetic whose pregnancy ended in demise in mid-trimester.

The third and highest MSAFP value belonged to a patient who on routine antenatal ultrasound showed an intrauterine foetal death with generalised foetal oedema. Foetal blood sampling prior to mid-trimester termination of pregnancy revealed the cause to be a primary rubella infection. (NB: This abnormal foetus was not recorded in the above-mentioned register.)

The median MSAFP values rises progressively with gestational age between 14 and 19 weeks and is in keeping with values established in clinical trials of the ABBOT AFP-Enzyme Immuno Assay Monoclonal kit shown in Fig 4[11], albeit at a slightly higher level.

In the study, there were 3 abnormal pregnancies excluded from the analysis as detailed in Table I.

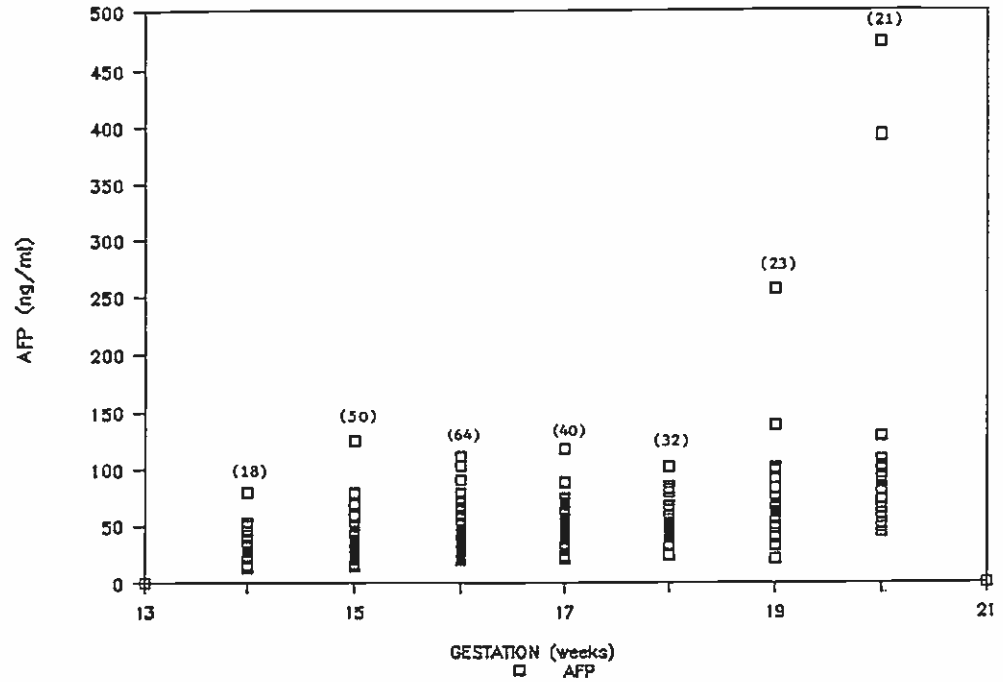
Case 1 was referred from the Maternal and Child Health Clinic for amniocentesis to screen for Down's syndrome because of her advanced maternal age. Case 2 was referred from a private maternity hospital for profuse bleeding per vaginum at 23+ weeks amenorrhoea associated with severe oligohydramnios. Chorionic villous sampling was done as foetal cord blood sampling was technically difficult. A male foetus with fused eyelids but otherwise grossly normal appearance spontaneously aborted at 24+ weeks gestation. Case 3 had an anencephalic foetus which was picked up on routine ultrasound screening.

DISCUSSION

As with any screening test, there is always a struggle with the equation between sensitivity (detection rate) and specificity.

Using maternal age as the only criterion for genetic amniocentesis, the majority of Down's syndrome births will be

Fig 2 - MSAFP Scatter
From 14 to 20 Weeks Gestation



Note : Figures within brackets indicate the number of values for each gestation

Fig 3 - Local Median MSAFP Values for 14 to 20 Weeks Gestation

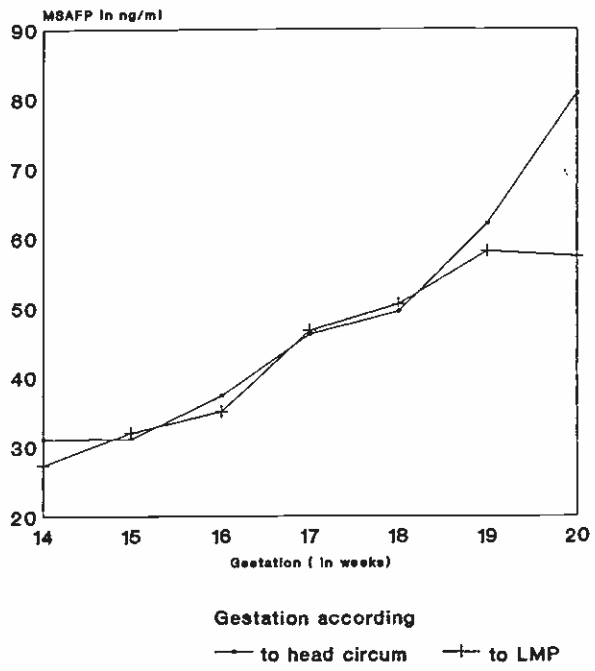
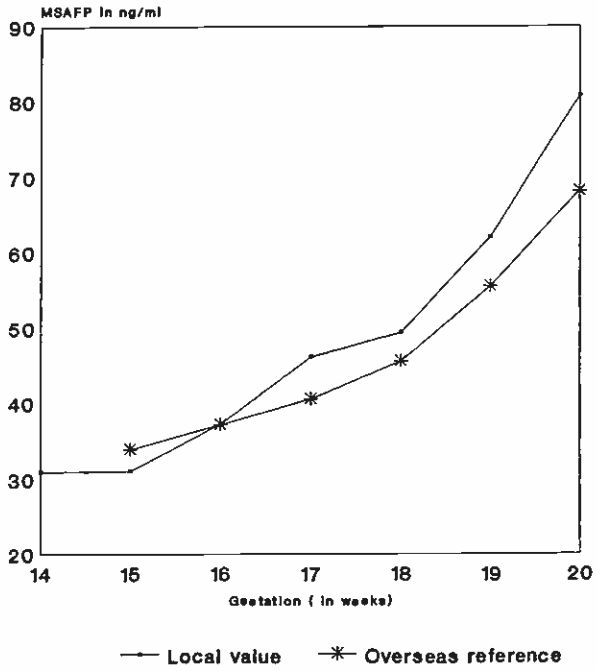


Fig 4 - Median MSAFP Values for 14 to 20 Weeks Comparing Local to Overseas Reference



missed. A screening tool independent of maternal age would help to identify a high-risk subset of the pregnant population in the younger mothers. However, in using MSAFP, both the sensitivity and specificity will depend on what is chosen as the abnormal AFP level.

An effective screening policy would be to offer an amniocentesis to all women above a certain age and, among the younger mothers, only to those with low MSAFP levels, using different cut-off levels depending on their maternal age. We were unable to retrospectively analyse the Down's syndrome pregnancies which had MSAFP done as there was only one patient (Case 1). However, several retrospective studies using various proposed screening policies for Down's syndrome using both maternal age and serum AFP have shown that it is more efficient than either alone[6-8].

Accurate gestational dating is critical for MSAFP interpretation[7]. It is established that ultrasound if done early is more accurate in determining the gestational age than estimating from LMP. The ultrasound parameter used by the authors is the head circumference of the foetus as we believe it

Table I
Abnormal Pregnancies Excluded from Study

	Age (yrs)	Karyotype	GSTLMP	MoM	GSTGHC	MoM
1.	39	47XY+21	16.4	1.31	16.1	1.22
2.	35	46XY	23.4	(106.9)	22.6	(106.9)
3.	25	46XY	22.0	(295.5)	-	-

GSTLMP : Gestation by last menstrual period
GSTGHC : Gestation by head circumference on ultrasound cephalometry
MoM : Multiple of median
( ) : Actual MSAFP value in ng/ml

to be more accurate than the biparietal diameter.

The risk of miscarriage attributable to amniocentesis is probably less than 1.5%[12] and is less than 0.5% in our hands (unpublished data). Let us assume a 0.5% foetal loss rate due to amniocentesis, and the cut-off risk of DS at amniocentesis as 1 in 256 (equivalent to 1 in 365 at birth which is the risk for a 35 year old mother) as an indication for an amniocentesis[13]. Then for every case of DS detected, it has been estimated that one foetus would be lost in the effort to prevent the birth of a DS infant.

Because AFP assays may be less precise when measuring low levels, strict laboratory quality control measures to maintain validity of both low and high serum AFP results are important. MSAFP levels should not be interpreted without accurate menstrual dates and, if necessary, foetal ultrasonography. When reporting the result of this test to the patient, the referring physician may include the risk for Down's syndrome based on maternal age and AFP level. However, the following comparative risk figures should also be included: 1) the risk of Down's syndrome at birth at age 35 (one in 365); 2) the risk associated with amniocentesis.

The screening policy proposed would require greater clinical and cytogenetic resources, but these costs would need to be considered in the light of reduced cost of special care for children with Down's syndrome.

ACKNOWLEDGEMENT

The authors would like to thank Abbott Laboratories (S'pore) Pte Ltd; Dr Ho Lai Yun and the staff of the Neonatology Department; Ms Tan Bee Yian of the Department of Research & Evaluation, Ministry of Health; Ms Lee Sook Leng, Dr. A Aziz Ali and A/Nurse Suprapti Bte M Salleh of the Department of O&G for their assistance.

This study was supported by Grant No. BM/87/02 from the Singapore Science Council Research and Development Assistance Scheme.

REFERENCES

1. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosome abnormalities. Am J Obstet Gynecol 1984;148:886-91.
2. Ferguson-Smith MA, Yates JRW. Maternal age specific rates for chromosome aberrations and factors influencing them: report of a collaborative European study on 52,965 amniocenteses. Prenat Diagn 1984;4:5-44.
3. Cuckle HS, Wald NJ, Linderbaum RH. Maternal serum alpha-fetoprotein measurements: a screening test for Down's syndrome. Lancet 1984;i:926-9.
4. Fuhrmann W, Wendt P, Weitzel HK. Maternal serum-AFP as screening test for Down's syndrome. Lancet 1984;ii:413.
5. Murday V, Slack J. Screening for Down's syndrome in the North East Thames Region. Br Med J 1985;291:1315-8.
6. Hershey DW, Crandall BF, Perdue S. Combining Maternal Age and Serum Alpha-Fetoprotein to predict the Risk of Down's Syndrome. Obstet Gynaecol 1986;68:177-80.
7. Cuckle HS, Wald NJ, Lindenbaum RH. Screening for Down's syndrome using serum alpha-fetoprotein. Br Med J 1985;291:349.
8. Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987;94:387-402.
9. Report of the United Kingdom Collaborative Study on Alpha-Fetroprotein in Relation to Neural-Tube Defects: Maternal serum alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Lancet 1977;i:1323-32.
10. Crandall BF. Second Trimester Maternal Serum Screening to identfy Neural Tube Defects. In: Kirkpatrick AM, Nakamura RM. eds. Alpha-fetoprotein, Laboratory Procedures and Clinical Applications. New York: Masson Publishing, 1981:93-105.
11. Abbott Laboratories. Abbott AFP-EIA Monoclonal. Information for Physicians. 1986.
12. Second report of the UK collaborative study on alpha-fetoprotein 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:652-62.
13. Ferguson-Smith MA. Prenatal chromosome analysis and its impact on the birth incidence of chromosome disorders. Br Med Bull 1983;39:355-64.