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Normal Values of Second Trimester Maternal Serum Alpha-Fetoprotein in Thai Pregnant Women

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ABSTRACT

Objective To establish the normal values of second trimester maternal serum alpha-fetoprotein in Thai pregnant women.

Design Prospective descriptive study.

Setting Department of Obstetrics and Gynaecology, Siriraj Hospital.

Subjects During October 1992 and September 1993, pregnant women regardless of age and in their second trimester were recruited to the Siriraj Maternal Serum Alpha-Fetoprotein (MSAFP) Screening to detect Down syndrome and Neural Tube Defect.

Results From 2,485 women counseled, 770 were screened for serum alpha-fetoprotein. Amniocentesis was performed in 75 women age over 35 years. There were 1 neural tube defect, 3 stillbirths and 4 sets of twins in women with high MSAFP. One case of Down syndrome was found in a mother who was counseled but decided not to join the programme.

Conclusion The result of this study has firmly established the normal values of MSAFP in Thai pregnant women, from 15-21 weeks' gestation to detect common birth defects (DS and NTD) and women at increased risk of obstetric complications. Physician should be aware of the benefits of both high and low MSAFP screening not only for the prenatal detection of birth defects, but also for early identification of women at an increased risk of obstetric complications.

Key words : second trimester, maternal serum alpha-fetoprotein

Down syndrome (DS) is the most common and best known chromosomal abnormalities in man. It is also one of the most serious malformation syndrome causing moderate to severe mental retardation and many associated physical anomalies affecting the heart, gastrointestinal tract, eye and ear. The incidence of Down syndrome is 1 in 800 births (varying from 1 in 660 to 1 in 1,000 births worldwide). The aetiology of DS is chromosomal disorder being Trisomy 21 (95 percent), translocation (4 percent) and mosaic (1 percent).[1,2] In the first year of life the use of health services is about double that of unaffected infants, this is also in accordance to the 10-year retrospective study at Genetics Unit, Department of Paediatrics, Siriraj Hospital Medical School (from 1977-1986).[3] The main burden of care, however, arises from the fact that individuals with Down syndrome are completely dependent on others and require considerable personal supervision throughout their lives. Moreover, the burden is shared heavily by the family and the society as a whole.[4,5]

Until two decades ago, 50 percent of babies with DS were born to mothers over 35, but recent data document that only about 5-20 percent are now being born to women in the 35 and over age group.[6-8] The average maternal age to the birth of a DS is about 34 years. This is also similar to the 10 year retrospective study at Siriraj Hospital (1977-1986), in which the average maternal age was 32 years. Though the risk is lower at early maternal ages, there are many more births at the younger maternal ages that the absolute number of DS babies born to young mothers is quite high. (Fig. 1) In fact this number approaches 95 percent of mother under 35 years of age in certain report.[3,9-11]

In addition, maternal serum alpha-fetoprotein (MSAFP) has been used for many years to screen for neural tube defects (NTD) in western countries.[7,12-16] Recently, an association between low MSAFP levels and fetal chromosomal abnormalities has been observed and thoroughly documented. It has been postulated that maternal screening for neural tube defects could be used not only to look for high AFP levels which indicate risk of a neural tube defect, but also for low values to identify prospectively fetuses with Down syndrome. The normal values of alpha-fetoprotein

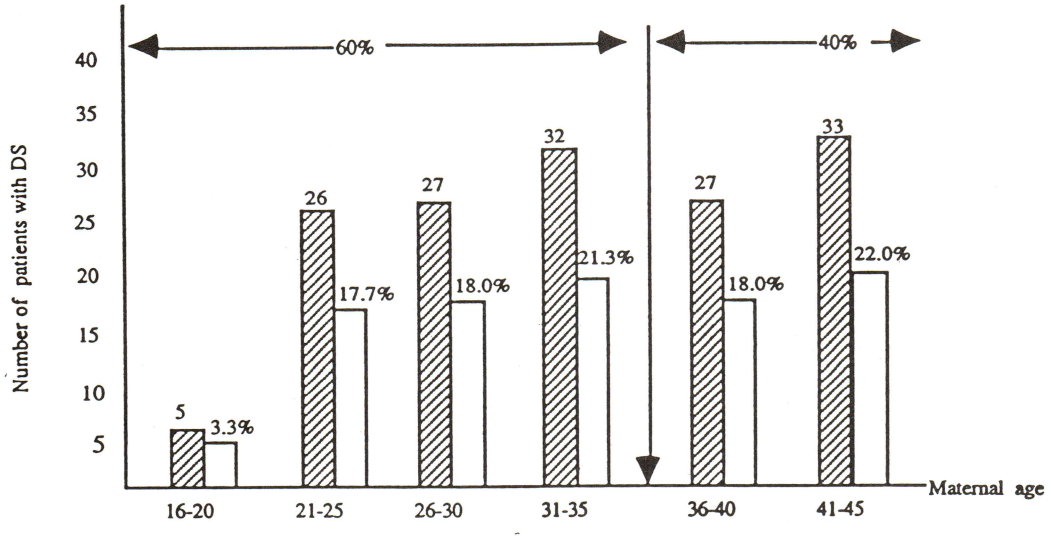

Fig. 1. Retrospective study of DS, Siriraj Hospital (1977-1986), total 150 (unpublished data).

must also be adjusted for gestational age, maternal weight, and maternal age.[17-20]

Women 35 years of age and older at the time of delivery have routinely been offered prenatal diagnosis for chromosomal abnormalities in western countries. However, women less than 35 years old have not been offered prenatal diagnosis routinely because of the relatively low risk of having a child with a chromosomal abnormality, limited laboratory facilities and the risks associated with the procedure. In United States, the present screening policy for chromosomal anomalies in the fetuses of women over 35 years of age is estimated to identify only 10-20 percent of all DS pregnancies. Using low MSAFP levels to screen mothers under 35 years of age would be expected to identify an additional 20-25 percent of cases.[21-23] Thus, the combined approach of MSAFP screening in pregnant women < 35 years old and amniocentesis in women ≥ 35 could be expected to identify up to 50 percent of all children with Down syndrome prior to 20 weeks of gestation.[24-26]

In conclusion, maternal serum AFP is not a diagnostic test for Down syndrome, but MSAFP may be used in women of all ages in conjunction with the maternal age to estimate the likelihood of high-risk pregnancies associated with a Down syndrome fetus or neural tube defects. MSAFP is, therefore, very useful to improve outcome of pregnancies.[27-29] The objective of this study was to establish the normal values of second trimester maternal serum alpha-fetoprotein in Thai pregnant women.

Materials and Methods

Pregnant women, regardless of age and in their second trimester (15-21 weeks' gestation), were recruited on a daily basis at the antenatal clinic, Obstetrics and Gynaecology department. Educational materials regarding the pilot project were distributed and video titled "Siriraj Maternal Serum Alpha-fetoprotein Screening to detect Down syndrome and Neural Tube Defect" was shown to a group of 20-30 pregnant women, twice daily. A questionnaire was given to pregnant women to be completed and returned after viewing of the video, or to return at the next antenatal care (ANC) visit after discussion with their husbands. Trained counsellors, consisted of medical geneticist, obstetrician, scientist, nurses and social worker, were on hand to answer any questions on a daily basis. Pregnant women were divided into two groups after video viewing and counseling session. Pregnant women under age 35 were given appointment to return for blood collection according to calculated date of gestational age. The gestational age was determined by last menstrual period (LMP) combined with fundus examination by the obstetricians. Pregnant women were encouraged to bring their husbands along for the next visit if they need more information to assist in the decision whether to join the programme, since the pilot project was on the voluntary basis. Informed consent was signed prior to joining the programme and a sticker was placed on the ANC form of the pregnant women. Weight was recorded prior to blood collection.

Pregnant women age over 35 were offered amniocentesis and appointment given to attend Genetics Clinic for individualized counseling for high-risk couple. If they decided to join the programme, the appointment would be given for MSAFP and amniocentesis according to the gestational age. They were referred to obstetricians for ultrasonography to determine accurate gestational age prior to amniocentesis. The chromosome study was done in the Genetics Laboratory at Department of Paediatrics. All

results were given by medical geneticist within 3 weeks. Abnormal results were discussed in great length with the couple. All pregnant women joining the programme had a file contained personal data e.g. maternal age, weight, previous history of maternal illness, previous history of birth defects or mental retardation, history of miscarriages, family history of genetic disorders, pre and post counseling questionnaire, signed consent form and laboratory data.

Blood specimens were collected daily with the assistance of the Department of Medical Technology. Specimens were sent to Genetics Laboratory at the Department of Paediatrics and maternal serum alpha-fetoprotein (MSAFP) were tested using Abbott AFP-EIA kit. The high and low MSAFP will be determined by comparison with data from United States and Singapore in the initial study. After we obtained mean MSAFP in each gestational age we used our own data for determination. The risk of Down syndrome in relation to maternal age during second trimester was calculated according to the methods suggested by the New England Regional Genetics Group using data reported by Hooke[30,31] and Haddow.[32,33]

Information and counseling were provided to the couple throughout the project to assist in making informed choices about whether to proceed with the programme, to understand the results and the options available. (Fig. 2)

Results

We have studied the maternal serum alpha-fetoprotein (MSAFP) in 770 Thai pregnant women (from 2,485 women counseled) and amniocentesis in 75 women over 35 years of age from October 1992 to September 1993. We summarized the

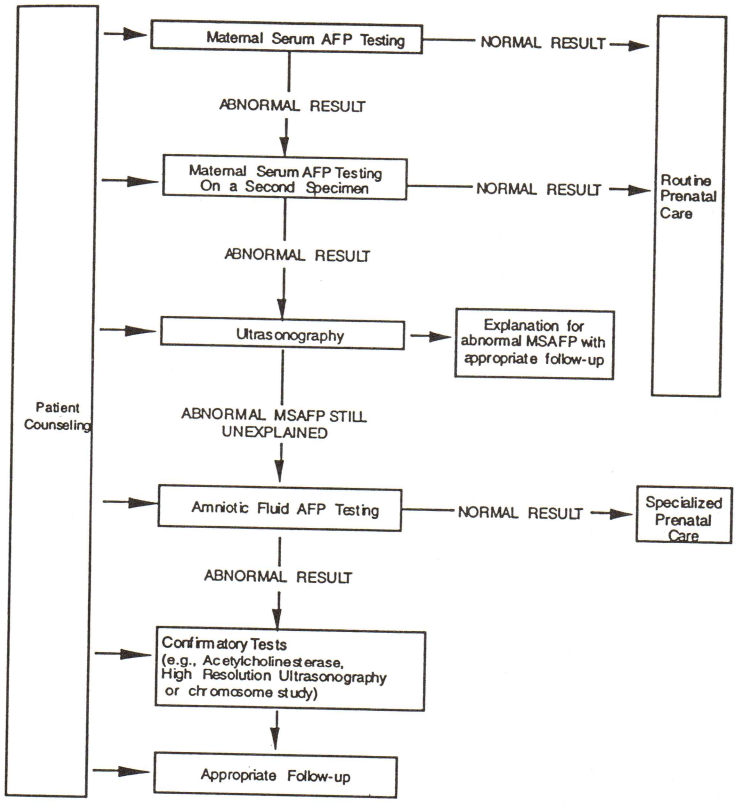

Fig. 2. Alpha-Fetoprotein Prenatal Testing Protocol.

results as follow :

Fig. 3 shows the distribution of number of pregnancies by gestational age from 15-21 weeks from which MSAFP was obtained based on LMP and ultrasonography. There are 66 pregnancies at 15 weeks' gestation, 115 pregnancies at 16 weeks' gestation, 126 pregnancies at 17 weeks' gestation, 133 pregnancies at 18 weeks' gestation, 120 pregnancies at 19 weeks' gestation, 112 pregnancies at 20 weeks' gestation, 66 pregnancies at 21 weeks' gestation.

Fig. 4 shows the distribution of MSAFP values from which the median for each gestational age is derived. As shown, at 15 weeks - the median MSAFP = 42.77 ng/mL ; at 16 weeks - the median MSAFP = 41.59 ng/mL ; at 17 weeks - the median MSAFP = 51.42 ng/mL ; at 18 weeks - the median MSAFP = 57.41 ng/mL ; at 19 weeks - the median MSAFP = 71.75 ng/mL ; at 20 weeks - the median MSAFP = 84.79 ng/mL ; at 21 weeks - the median MSAFP = 104.86 ng/mL.

Fig. 5 shows the weighed log-linear regression of MSAFP medians. After obtaining the median values, these will be adjusted using the following formula : Regression = 10 [(b + (mxGA)] , where intercept b = Y-mx ; slope m = N/D ; GA = gestational age. At 15 weeks where the median MSAFP = 42.77 ng/mL, the regressed MSAFP median = 37.32 ng/mL. At 16 weeks where the median MSAFP = 41.59 ng/mL, the regressed MSAFP median = 43.90 ng/mL. At 17 weeks where the median MSAFP = 51.42 ng/mL, the regressed MSAFP median = 51.64 ng/mL. At 18 weeks where the median MSAFP = 57.41 ng/mL, the regressed MSAFP median = 60.75 ng/mL. At 19 weeks where the median MSAFP = 71.74 ng/mL, the regressed MSAFP median = 71.46 ng/mL. At 20 weeks where the median MSAFP = 84.79 ng/mL, the regressed MSAFP median = 84.06 ng/mL. At 21 weeks where the median MSAFP = 104.82 ng/mL, the regressed MSAFP median = 98.89 ng/mL. From this data, the median MSAFP increases 17.6 percent.

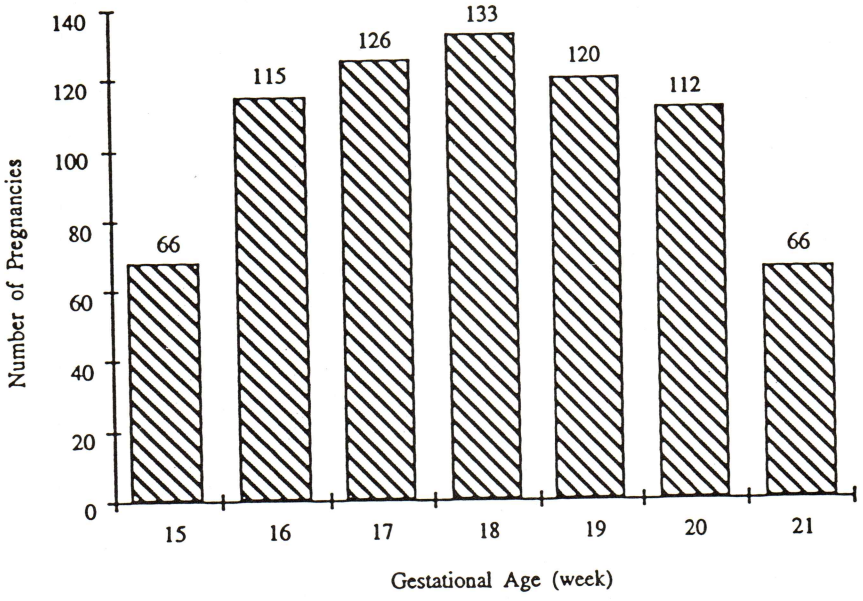

Fig. 3. Distribution of number of pregnancies by gestational age from 15-21 weeks for which MSAFP was obtained based on LMP and ultrasonography.

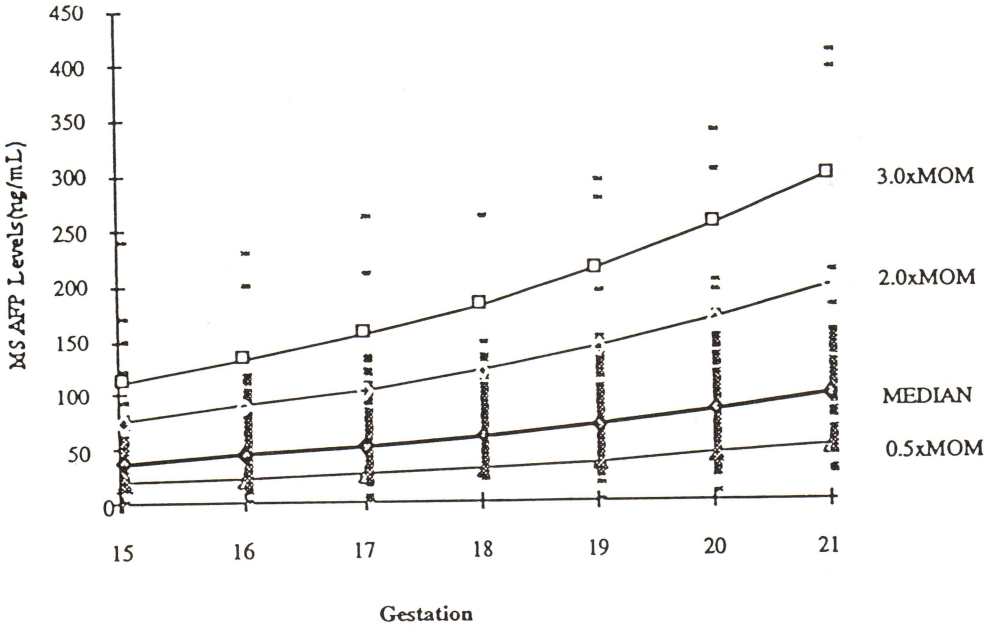

Fig. 4. Distribution of MSAFP values from which the median for each gestational age is derived.

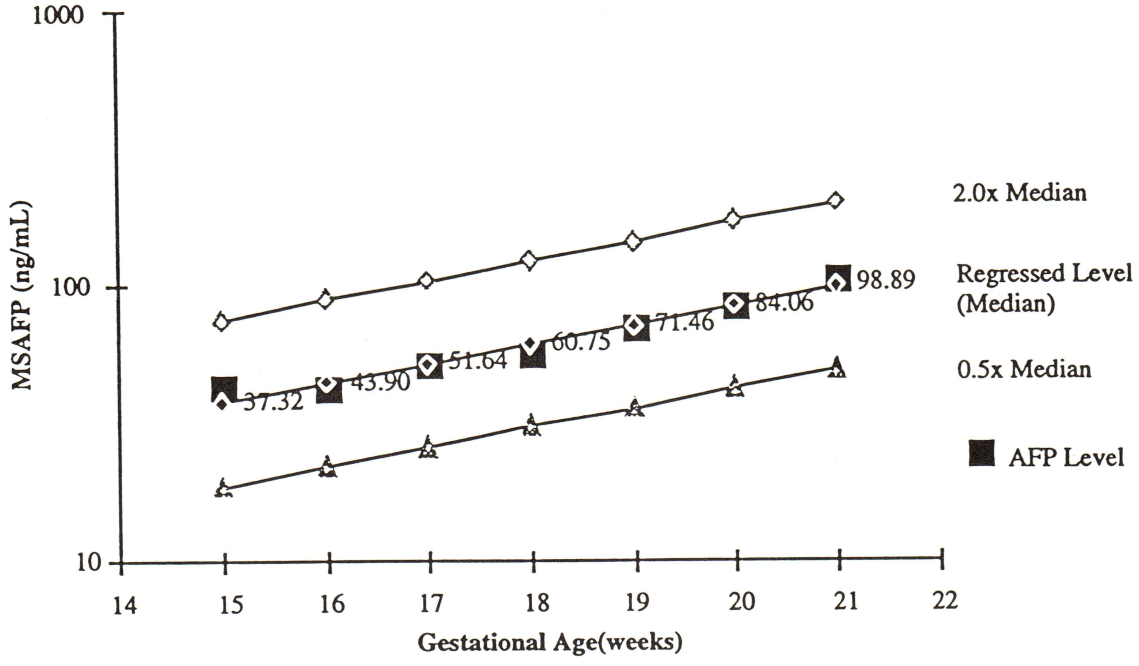

Fig. 5. Weighed log-linear regression of MSAFP medians using regression = 10 [(b + (mxGA)], where intercept b = Y-mX ; slope m = N/D ; GA = gestational age (from slope and intercept calculation).

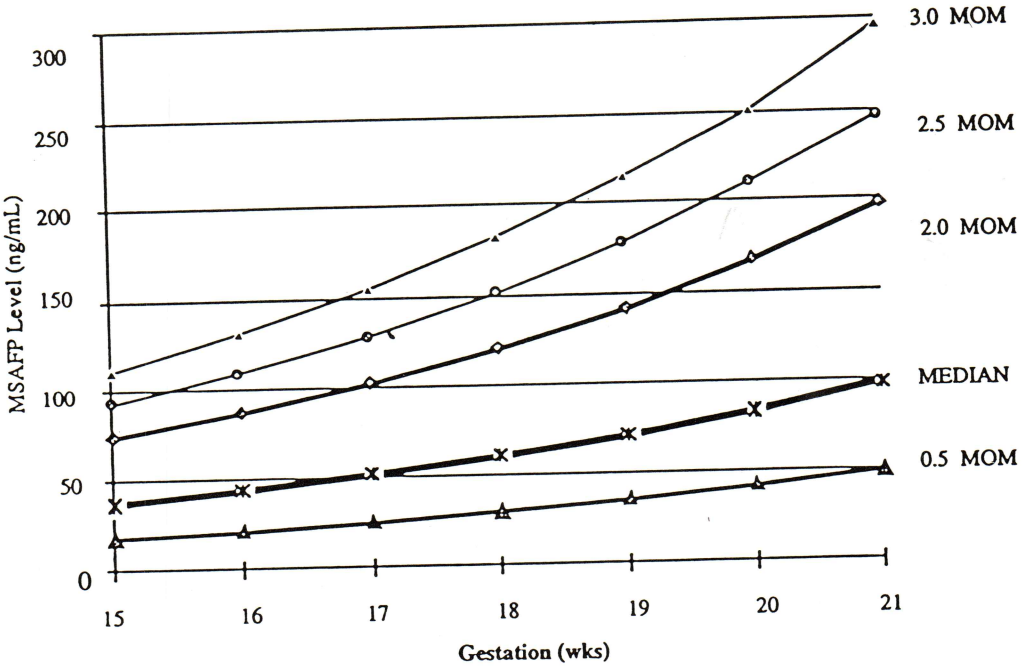

Fig. 6. MSAFP levels expressed in multiples of the median (MoM).

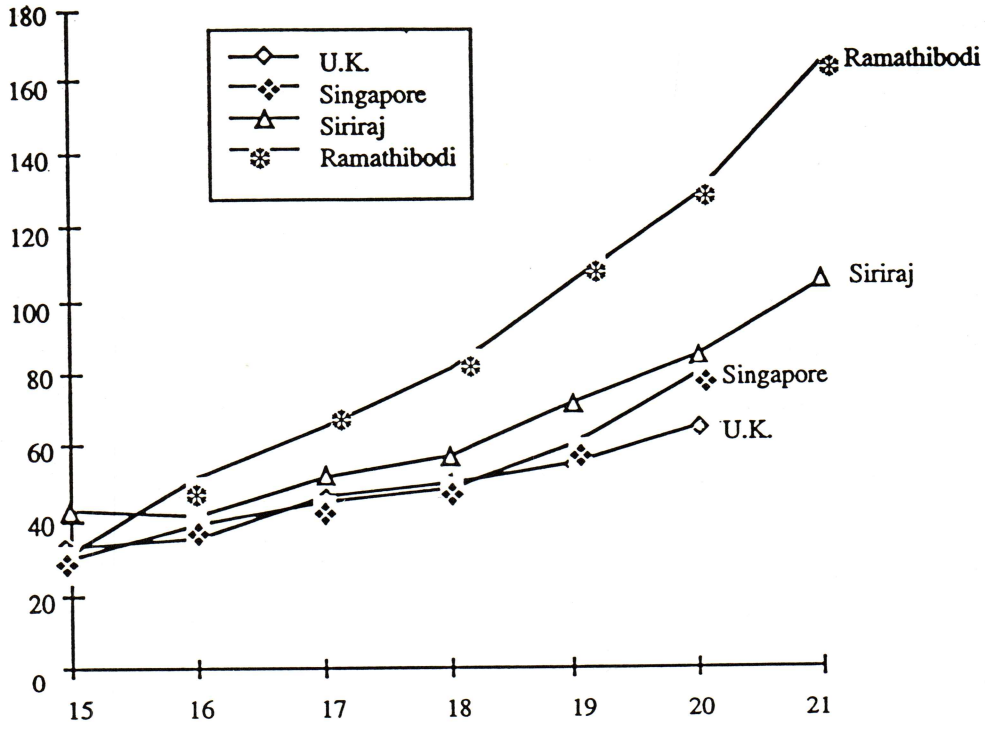

Fig. 7. Median MSAFP values comparing local to overseas references.

Table 1. High MSAFP using cut-off > 2.5 MoM

	Pregnancies
Gestation > 21 wks	11
Gestation, 15-21 wks, normal U/S	3
Repeated MSAFP, normal	4
Twins pregnancy	4
Lost follow-up	3
Placental separation	2
Spontaneous abortion (fall from bridge)	1
Stillbirth	1
Neural tube defect (Anencephaly)	1
Total	**30**

Table 2. Low MSAFP using cut-off < 0.5 MoM

	Pregnancies
Maternal age < 35 yrs.	
Deferred amniocentesis due to gestation over 21 wks.	6
Underwent amniocentesis	5
Refused amniocentesis (unknown reason)	1
Non-pregnancy	1
Maternal age ≥ 35 yrs.	
Underwent amniocentesis	14
Refused amniocentesis	1
Total	**28**

Table 3. Comparison of studies (local and overseas)

	Ramathibodi Hospital[34]	Siriraj Hospital	Singapore[35]
Total cases studied	150	770	362
Gestation age (wk)	13-23	15-21	14-20
Method of confirmation (LMP, PE, U/S)	U/S	LMP, PE, U/S	LMP, U/S
Maternal age	22-34	17-45	
Objectives for screening	NTD	NTD, DS	DS
Method used	RIA (Amersham)	EIA (Abbott)	EIA (Abbott)

Fig. 6 shows the MSAFP in 770 Thai pregnant women in multiple of the median (MoM). The median MSAFP 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.

Fig. 7 shows median MSAFP values comparing local to overseas reference. As shown, the MSAFP (MoM) values from United Kingdom, Singapore and Siriraj Hospital are consistent and in keeping with values established in many reports, including the U.S.A.

Discussion

Prenatal screening for neural tube defects using maternal serum alpha-fetoprotien (MSAFP) has been extensively used in the United Kingdom[14] and, to a lesser extent in the United States.[27] Elevated MSAFP were also predictive of gastroschisis, omphalocoele, cystic hygroma, congenital nephrosis, poor pregnancy outcome and other maternal and fetal complications. In 1984, Merkatz reported association of low MSAFP with Down syndrome and other chromosomal abnormalities.[36] There have been numerous studies in U.K. and U.S.A regarding the MSAFP screening and normal values of MSAFP in each gestation had been established. However, there was no data available from Thai pregnancies except the study by Ramathibodi hospital in 1984-1985.[34] Recent study from Singapore demonstrated local norms of MSAFP from oriental population.[35]

In establishing local norms of MSAFP in a population, each laboratory must establish its own reference data using samples obtained from the population to be screened. Most authorities recommend measuring AFP in 100 samples for gestational weeks 15 through 20 for calculation of a median.

Our study in 770 pregnancies firmly establishes the normal values of MSAFP in Thai pregnant women, from 15-21 weeks' gestation which can be used for routine screening of pregnant women to detect common birth defects (DS and NTD) and to improve outcome of pregnancies. However, in using MSAFP, both the sensitivity and specificity will depend on what is chosen as the abnormal AFP level. In this study, we use a cut-off > 2.5 MoM for high MSAFP and cut-off < 0.5 MoM for low MSAFP (Table 1, 2). The MSAFP values between 2-2.5 MoM are considered borderline high and need to be repeated. Underestimation of gestational age is a common cause of high MSAFP results than fetal abnormalities. Multiple gestation is also a cause of high MSAFP levels and is frequently identified through MSAFP screening. Most common reason for low MSAFP results is an overestimation of gestational age. It is also possible that the fetus has a chromosomal abnormality other than Down syndrome or the patient is not pregnant. MSAFP levels of normal pregnancies may also be in the low range.

Accurate gestational dating is critical for MSAFP interpretation. It is established that ultrasonography is more accurate in determining the gestational age than estimating from LMP. However, with limited resources we were able to combine LMP and PE (by fundus examination) and in at least 20 percent ultrasonography was done for accurate gestation.

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.[14] The median AFP values 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.

A comparison of MSAFP (MoM) from different studies (local and overseas) is demonstrated in Fig. 7 and Table 3. Due to a rather small sample size (770 pregnancies) we are unable to detect the most common birth defect (Down syndrome, incidence 1 per 800). However, we are able to firmly establish the normal values of second -trimester MSAFP of Thai pregnant women.

MSAFP screening should be voluntary and offered as part of routine obstetrical care. Combination of maternal age and MSAFP can be used to identify pregnant women under age of 35 years who have increased risk of having fetus with Down syndrome. Counseling should be provided prior to the MSAFP screening and should be informative and non-directive. Quality control should be an important part of MSAFP programme. Women who have high MSAFP and whose fetus does not have a demonstrable abnormality by either level II ultrasound or karyotyping from amniotic fluid, continue to have an increased risk for having a fetus with major congenital malformation and poor pregnancy outcomes, such as perinatal deaths and low birthweight.

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