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OBSTETRICS

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

Pornswan Wasant MD,* Jeerasak Manussakorn MD,**
Sujin Kanokpongsakdi MD,** Pornpimol Ruengwutilert MD,**
Nattee Raksadawan MD,** Supaporn Wattanaweeradej MSc,*
Wattana Boonyawit BSc,* Nongyao Satrasook BA,*
Nualanong Booncharunsilp MSc,* Chotipa Sakulsingharoj BSc,*
Lawun Inngarm BSc,* Sukanya Taweesri BSc.*

* *Department of Paediatrics,*

** *Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Thailand*

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

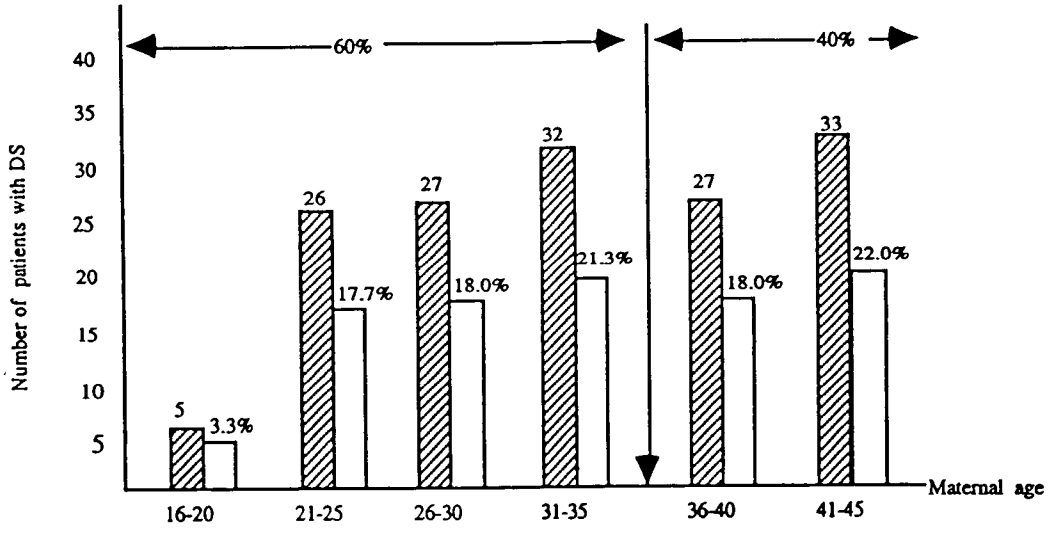

Fig. 1. Retrospective study of DS, Siriraj Hospital (1977-1986), total 150 (unpublished data).

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

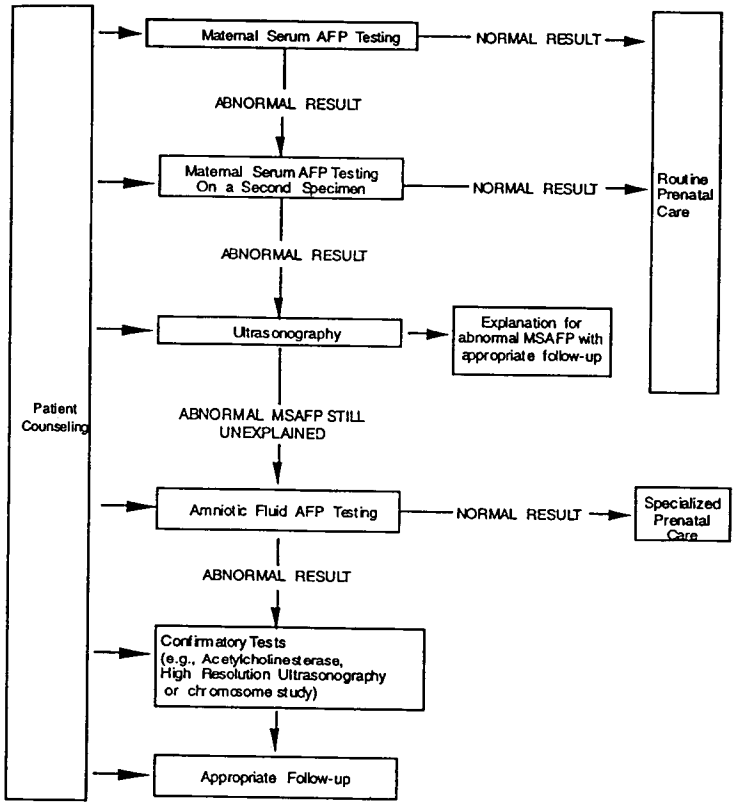

Fig. 2. Alpha-Fetoprotein Prenatal Testing Protocol.

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.

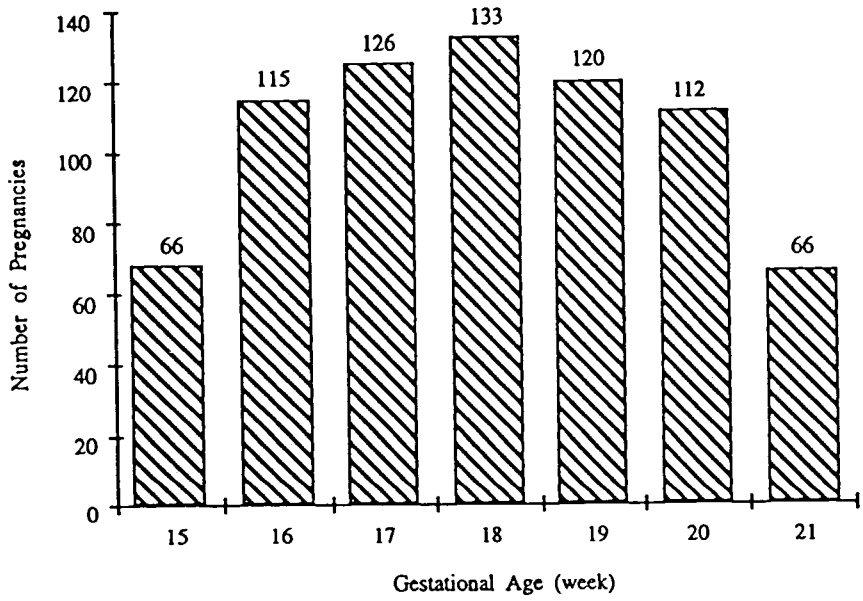

Fig. 3. Distribution of number of pregnancies by gestational age from 15-21 weeks for which MSAFP was obtained based on LMP and ultrasonography.

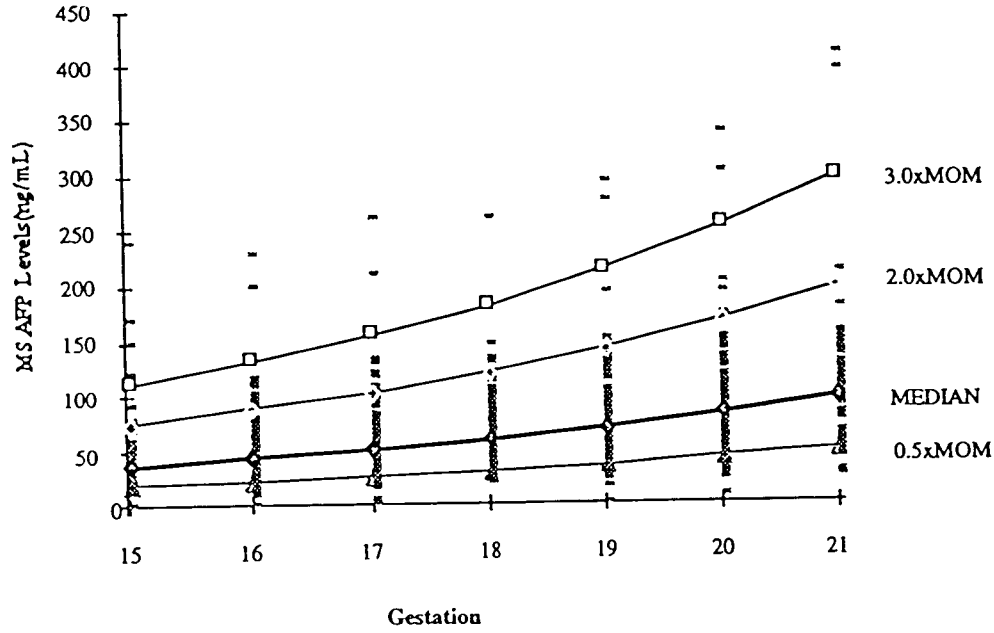

Fig. 4. Distribution of MSAFP values from which the median for each gestational age is derived.

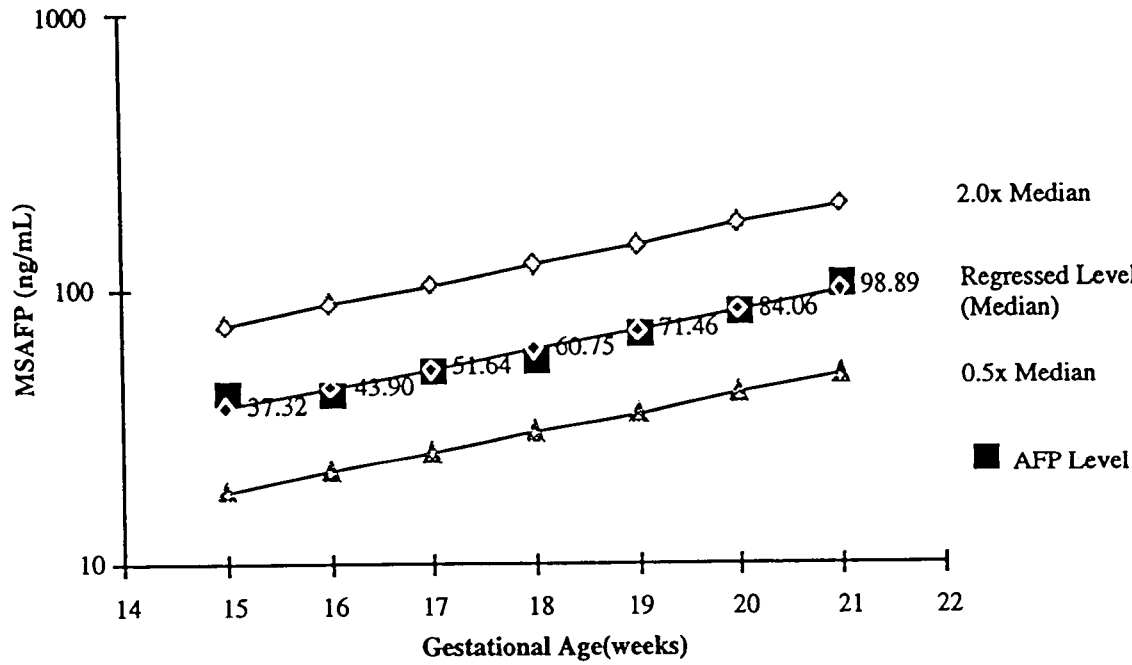

Fig. 5. Weighed log-linear regression of MSAFP medians using regression = 10 [(b + (mxGA)], where intercept b = Y-mX ; slope m = N/D ; GA = gestational age (from slope and intercept calculation).

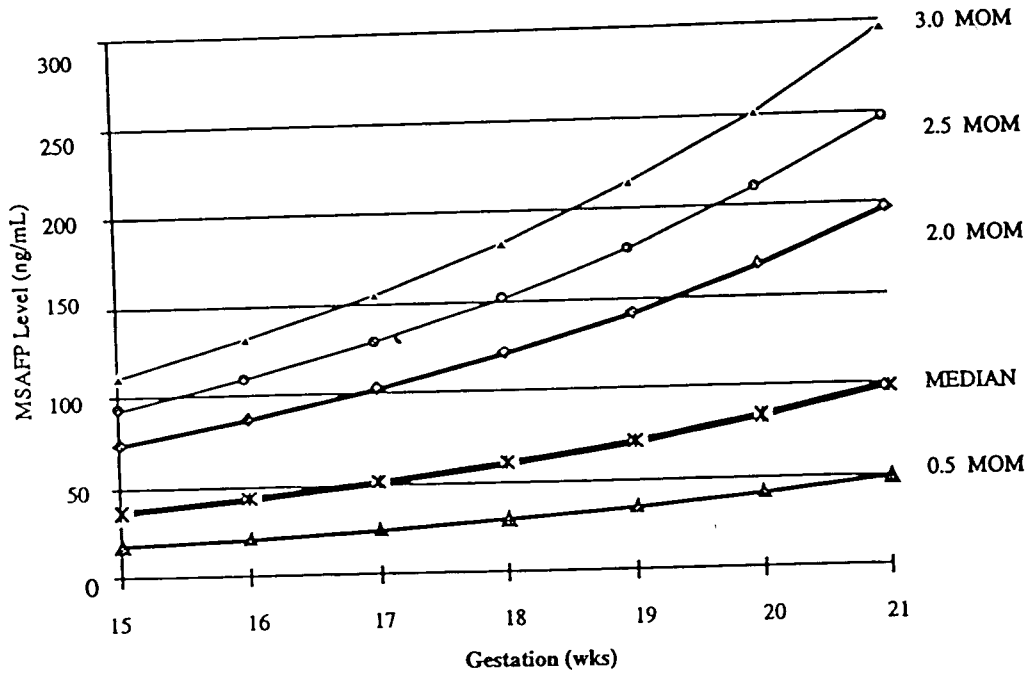

Fig. 6. MSAFP levels expressed in multiples of the median (MoM).

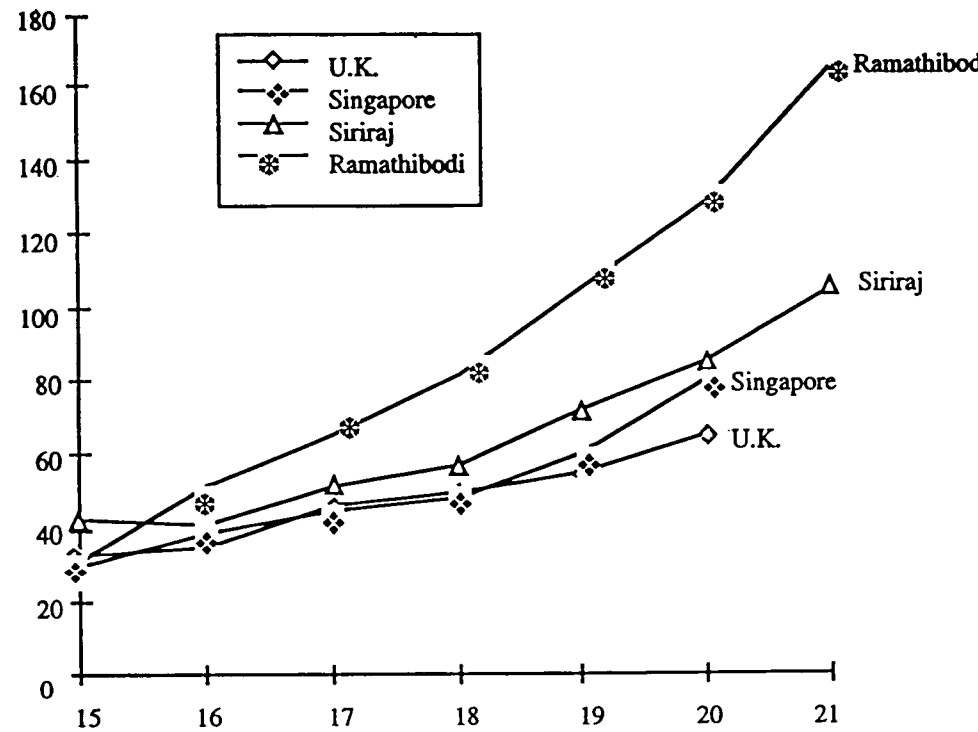

Fig. 7. Median MSAFP values comparing local to overseas references.

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.

Acknowledgement

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Royal Thai College of Obstetricians and Gynaecologists

MEETING AND COURSES IN 1997

21-22	APRIL	REFRESHER COURSE (to be held at the Sol Twin Towers Hotel)
12-14	MAY	RESEARCH METHODOLOGY (to be held at the Siriraj Hospital)
15-17	OCTOBER	XII th SCIENTIFIC AND ANNUAL RTCOG MEETING (to be held at the Central Plaza Hotel, Bangkok)

Further details can be obtained from :

Dr. Kobchitt Limpaphayom

Department of Obstetrics and Gynaecology

Faculty of Medicine, Chulalongkorn University

Chulalongkorn Hospital, Bangkok 10330, Thailand

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OBSTETRICS

Prenatal Diagnosis of Severe Thalassemia Syndrome in Maharaj Nakorn Chiangmai Hospital

Supatra Sirichotiyakul MD,*
Chanane Wanapirak MD,*
Pannee Sirivatanapa MD,*
Torpong Sa-nguansermsri MD,**
Ratanaporn Sekararithi BA,*
Apiradee Tuggapicthitti BSc,*
Kulaya Payu BSc,**
Areerat Panyakhew BSc.**

* *Department of Obstetrics and Gynaecology ,*

** *Department of Paediatrics, Faculty of Medicine, Chiangmai University, Chiangmai, Thailand*

ABSTRACT

Objective To describe prenatal diagnosis programme for prevention and control of severe thalassemia syndrome which was successfully carried out at Maharaj Nakorn Chiangmai Hospital.

Design A prospective descriptive study.

Setting Maharaj Nakorn Chiangmai Hospital.

Subjects and methods The programme included : 1) screening of pregnant women for thalassemia carriers in order to identify couples at risk of having baby with severe thalassemia syndrome, 2) prenatal diagnosis by serial ultrasonography or fetal blood sampling under ultrasound-guided cordocentesis in risk couples, 3) analysis of fetal blood under HPLC (High Performance Liquid Chromatography) or haemoglobin electrophoresis, 4) genetic counseling of couples with affected fetus and discontinuing pregnancy.

Results From 13th September 1994 to 1st August 1995, 3,310 pregnant women were screened for carriers of important thalassemia by EOFT (Erythrocyte Osmotic Fragility Test) and 1,000 cases (30.2%) gave abnormal results. Among them 115 couples were at risk, 87 of them obtained prenatal diagnosis and 19 of 87 fetuses were severe thalassemia syndrome and were terminated.

Conclusion Prenatal diagnosis programme for thalassemia is an obstetric role in prevention and control of the disease.

Key words : prenatal diagnosis, thalassemia

Thalassemia is the most common haematologic genetic disease in Thailand. About 500,000 Thai people are affected and more than 15 million Thais are carriers. By calculation of abnormal gene prevalence, the couples at risk for having an affected child are 50,000 pregnancies a year. The common type of thalassemia are homozygous beta-thalassemia, beta-thalassemia Hb E disease, Hb Bart's hydrops fetalis and Hb H disease. The affected persons will have low quality of life (blood transfusion, gall stone, etc) and the mothers who have hydropic fetus will suffer from obstetric complications such as pre-eclampsia, antepartum haemorrhage and postpartum haemorrhage. Prevention of the new case is an obstetrician's role to control the disease.

In prevention and control programme of thalassemia, genetic counseling and carrier detection followed by prenatal diagnosis of the risk couple should be in the step. Maharaj Nakorn Chiangmai hospital is the largest tertiary care centre of Northern Thailand which has to face the problem of thalassemia due to a high prevalence of abnormal gene.

Carrier detection of thalassemia in asymptomatic patients can be achieved by different methods such as red blood cell (RBC) indices (MCV), haemoglobin electrophoresis, PCR (polymerase chain reaction) technique and erythrocyte osmotic fragility test (EOFT). In this study, EOFT was chosen to screen the patients because it is a rapid, simple procedure and not expensive or time consuming, and suitable for mass screening.[1,2]

Materials and Methods

Laboratory Tests

Erythrocyte Osmotic Fragility Test (EOFT) is a rapid and simple method for determination of RBC osmotic fragility,[3] using hypotonic solution (0.36% NaCl or glycerine saline solution). At a specific time (90 seconds), rate of haemolysis of normal erythrocyte is more than 50% while in cases of abnormal erythrocyte like thalassemia and thalassemia carriers haemolysis of erythrocyte is less than 50%. In this study, "less than 60% haemolysis at 90 seconds" was used as cut off point of abnormal erythrocytes to decrease false negative test. Laboratory methods for this study was

(1) Reagents : 0.45% glycerine saline solution (0.45% GSS), pH 7.4

(2) Specimens : Venous blood collected in EDTA, centrifuge at 3,000 rpm for 5 minutes, then remove supernatant and collect RBC for the test

(3) Procedure :

(a) Add 20 μl of RBC to 20 ml of distilled water and mix together. When there is complete haemolysis, set a spectrophotometer to record its absorbance at 620 nm as 0 (zero)

(b) Add 20 μl of RBC to 20 ml of 0.45% GSS and mix together. Record the absorbance every 15 seconds until 120 seconds, assume that absorbance at 0 and 15 seconds = 100 (No haemolysis)

$$\% \text{ haemolysis} = \frac{\text{Absorbance at 15 sec} - \text{Absorbance at 90 sec}}{\text{Absorbance at 15 sec}} \times 100$$

If the laboratory test was abnormal (EOFT < 60%, at 90 sec), the sample was further tested for HbA_2 level (by microcolumn DEAE Sephadex A 50 chromatography) to differentiate the patient as alpha-thal 1 trait, beta-thal trait or Hb E trait. (Fig. 1)

Subjects

Prenatal diagnosis programme for prevention and control of severe thalassemia syndrome was conducted at the antenatal care clinic (ANC) of Maharaj Nakorn Chiangmai Hospital since 13th September 1994. The data was collected

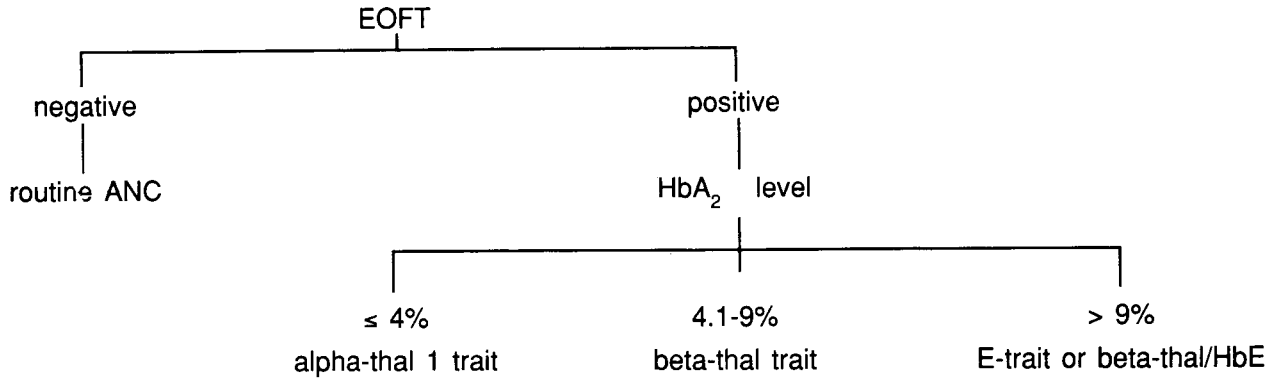

Fig. 1. Algorithm for thalassemia screening.

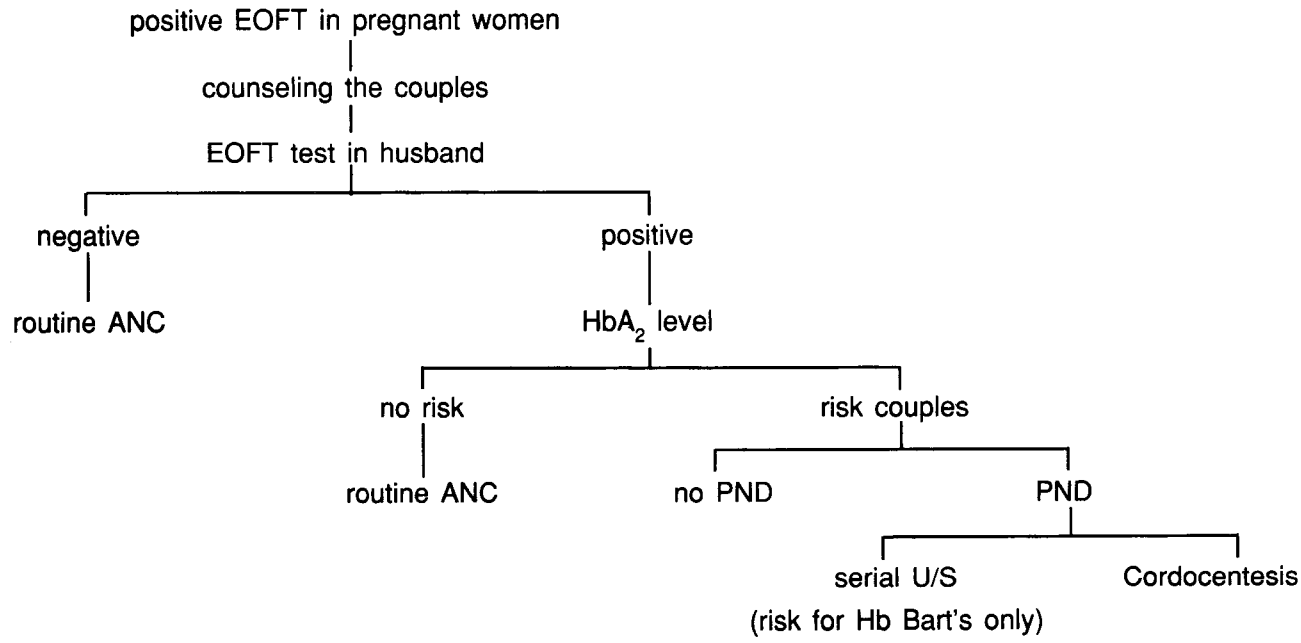

Fig. 2. Algorithm for counseling and management of risk couples.

from 13th September 1994 to 1st August 1995.

All pregnant women who first came to ANC received a paper counseling for thalassemia screening. Two ml of blood was collected and tested for EOFT followed by HbA_2 level in abnormal EOFT cases. Once the test was positive, the patient and her husband were asked to come for intensive counseling. The counseling included the screening tests in husbands, the chance to have an affected child and the options of prenatal diagnosis. (Fig. 2)

The risk couple for having an affected child was the couple who both were alpha-trait/both were beta-trait/or one was beta-trait while the other was Hb E trait. They may be risk for having Hb Bart's hydrops fetuses, homozygous beta-thalassemia or beta-thalassemia Hb E child depending on their types of carrier states.

After identifying and counseling the risk couples, prenatal diagnosis was offered. The couples who at risk for having Hb Bart's hydrops fetalis may choose prenatal diagnosis (PND) by either cordocentesis at 18-22 weeks or serial ultrasonography (every 2-3 weeks from 20-32 weeks). The couple who at risk for having homozygous beta-thalassemia or beta-thalassemia Hb E child were offered cordocentesis. The aim of serial sonography was an early detection of hydrops fetus which usually occurred in late 2nd or early 3rd trimester. Ultrasound findings included placental thickening (> 5 cm), fetal ascites, edematous skin, cardiomegaly and hepatomegaly. Cordocentesis was performed under ultrasound-guided, 2 ml of fetal blood was collected and analysed by HPLC technique (High Performance Liquid Chromatography) or Hb electrophoresis. The patients were followed up by routine antenatal care if the results of PND were negative for disease and pregnancy was terminaled if the fetuses were affected.

Pregnant women who have negative EOFT, or positive EOFT with negative EOFT husbands (no risk) and the couples who were assumed different kinds of carrier state (one was alpha-trait and the other was beta-trait or Hb E trait) were followed up by routine antenatal care.

Results

During the study period, there were 5,038 pregnant women attended antenatal care clinic, 3,310 women accepted for the screening and 1,000 gave abnormal EOFT (30.2%). Using HbA_2 level to group the patients, 494 were assumed alpha-thal 1 trait, 273 were beta-thal trait and 233 were Hb E trait or beta-thalassemia Hb E disease.

Five hundred and ninety-five couples came for intensive counseling and 593 husbands accepted the screening and gave positive EOFT 216 cases. After matching the result of pregnant women and her husbands, there were 115 risk couples for having thalassemia child ; 66 were at risk for Hb Bart's hydrops fetalis, 20 were at risk for homozygous beta-thalassemia and 29 were at risk for beta-thalassemia Hb E disease. Prenatal diagnosis was done in 87 couples ; 54 cases by cordocentesis and 33 cases (who were at risk for Hb Bart's hydrops fetalis) by serial ultrasonography. The indications for cordocentesis included risk for Hb Bart's hydrops fetalis 23 cases, risk for homozygous beta-thalassemia 13 cases and risk for beta-thalassemia Hb E 18 cases.

From this prenatal diagnosis programme 19 affected fetuses were detected. Analysis of fetal blood from cordocentesis showed 15 affected cases ; 5 cases were homozygous beta-thalassemia, 2 cases were beta-thalassemia Hb E disease and 8 cases were Hb Bart's. Serial ultrasonography could detect 4 hydrops fetuses

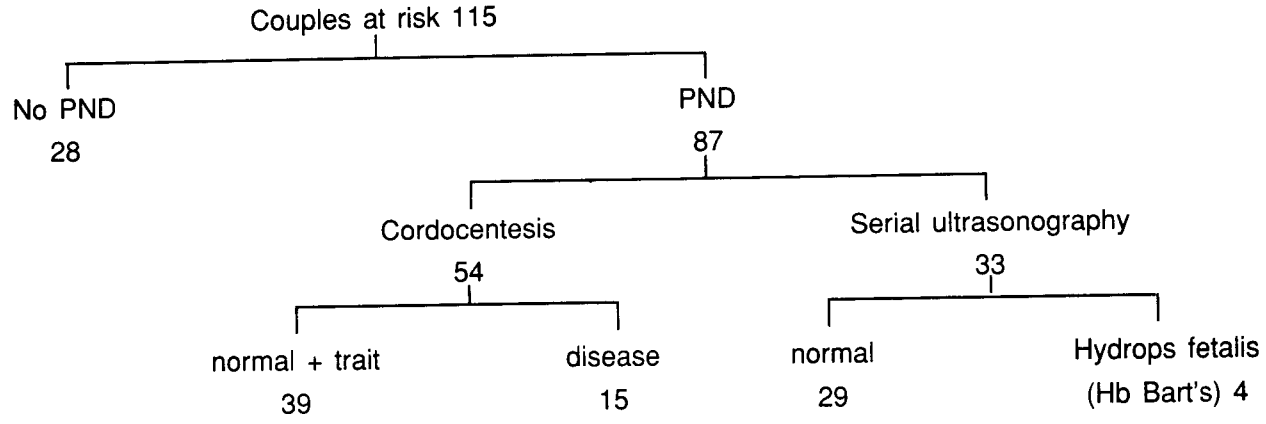

Fig. 3. Results of the programme.

who all were Hb Bart's (confirmed by fetal blood analysis). (Fig. 3)

Discussion

Thalassemia is a common haematological disease in Thailand. Prenatal diagnosis programme for severe thalassemia syndrome was conducted to prevent and control the disease. The programme included screening for thalassemia carriers in pregnant women to identify risk couples, prenatal diagnosis for risk couples and termination of pregnancy for affected fetuses.

EOFT was used to screen for thalassemia carriers because it is a simple, rapid, inexpensive and not time consuming procedure and suitable for mass screening. One technician can do up to 150 tests per day. Abnormal EOFT was found in 1) thalassemia and thalassemia carriers (both alpha-trait and beta-trait) 2) iron deficiency anaemia 3) sickle cell anaemia 4) chronic renal failure and 5) lead poisoning.[2-4] In this screening programme EOFT was tested in pregnant women who were healthy. We can exclude chronic renal failure and lead poisoning by history taking, physical examination and laboratory tests. It was assumed that no sickle cell anaemia in this study because of very low incidence in Thai population. Thus, when EOFT test was abnormal we have to differentiate thalassemia carriers, thalassemia and iron deficiency anaemia. Thalassemia can be diagnosed by clinical pictures, peripheral blood smears and Hb typing. Iron deficiency anaemia can be diagnosed by low haemoglobin concentration and abnormal laboratory tests (MCV, serum iron, TIBC). But in thalassemia carriers, they are not anaemia, no thalassemic facies and peripheral blood smears are usually normal. So, in this study we assumed that the study group were thalassemia carriers if they have abnormal EOFT and normal haemoglobin level. However we included both thalassemia and thalassemia carriers into the study group because both can transfer abnormal genes to the fetus.

"Less than 60% haemolysis at 90 seconds" was used as cut off point of abnormal erythrocytes in this study because a screening test should have low false negative rate. If we use lower cut off point, some thalassemia carriers will have normal EOFT and cannot be detected. However, for the cut off point used in this study some patients who were normal may had abnor-

mal EOFT (false positive test).

Flatz and Flatz studied one-step osmotic fragility test in 250 healthy Thai people and concluded that in the Thai group examined iron deficiency is rare and most increased osmotic indices (time to 50% haemolysis more than 90 seconds) not caused by beta-thalassemia were due to alpha-thalassemia 1.[1] We can detect beta-thalassemia trait and Hb E-trait by elevated HbA_2 level (> 4%) but in alpha-thalassemia 1 trait HbA_2 level are normal. When the patients had normal HbA_2 level, they might be alpha-thalassemia 1 trait or not. Due to the high prevalence of alpha-thalassemia 1 trait in Northern Thai people and Flatz and Flatz report, we assumed that "abnormal EOFT and normal HbA_2 level" patients were alpha-thalassemia 1 trait. This conclusion may not be used in other population because of the different prevalence. However, detection of alpha-thalassemia 1 gene in this group (by PCR technique) to determine false positive test of EOFT in detection alpha-thalassemia 1 trait is further studied.

Using only HbA_2 level has a pitfall. When HbA_2 level elevated the patients were assumed to be beta-thal trait despite they might be both alpha and beta trait. We found some couples that one was assumed to be beta-thal trait and the other was assumed to be alpha-thal 1 trait, so prenatal diagnosis was not offered and later the fetuses developed hydrops. From these cases we advise that PCR technique should be used to detect alpha-thal 1 gene in all abnormal EOFT results.

In our study, we screened 3,310 pregnant women and found 115 couples at risk for having an affected child. After counselling, prenatal diagnosis was done in 87 couples and 19 affected fetuses were diagnosed and terminated. From this study we can prevent 19 affected cases which would cost a lot of expenditure for treatment if pregnancy continue.

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OBSTETRICS

Prenatal Sonographic Diagnosis of Cystic Hygroma Colli

Theera Tongsong MD,
Chanane Wanapirak MD,
Wirawit Piyamongkol MD.

Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

ABSTRACT

Objective To evaluate the role of prenatal sonography in identifying characteristics of fetal cystic hygroma.

Design Case series.

Setting Department of Obstetrics and Gynaecology, Faculty of Medicine, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University.

Subjects A total of 12 fetuses with prenatal diagnosis of cystic hygroma were evaluated and followed.

Results All cases were correctly diagnosed sonographically during the first half of pregnancy. All showed asymmetric, multiseptate cystic masses (mostly thin-walled) at posterolateral aspects of the neck. Midline septation representing the nuchal ligament extending from the posterior neck outlined by bilateral cysts were identified in all cases and may be the most specific sign for diagnosis of cystic hygroma. Fifty-eight percent (7 cases) had already developed some degree of hydrops fetalis. Fifty-eight percent (7 cases) had oligohydramnios and only one case had polyhydramnios. Chromosome studies were done in 7 fetuses, 3 had chromosomal abnormalities, 45 X0 (2 cases) and trisomy 21 (1 case).

Conclusion This small series indicates that ultrasound had high predictive value in diagnosis of cystic hygroma. The most associated abnormalities were hydrops fetalis and oligohydramnios.

Key words : cystic hygroma, prenatal diagnosis, ultrasound

Cystic hygroma is a congenital malformation of the lymphatic system. It is the most common neck mass identified in the fetus. The incidence of cystic hygroma is not well documented. Reports ranged from 1 in 6,000 pregnancies to 1 in 120 pregnancies at risk for having this structural anomaly.[1-3]

The fetal lymphatic vessels drain into two large sacs lateral to the jugular veins. If the lymphatic and venous structures fail to connect, the jugular lymph sacs will be enlarged, resulting in the dilatation of the sacs into cystic hygromas of the posterior triangles of the neck which may lead to jugular lymphatic obstruction and hydrops fetalis.[4]

The cysts are characteristically found at the posterolateral region of the neck and are frequently divided by random, incomplete septa.[2] A dense midline septum extending from the fetal neck across the full width of the hygroma is found. The septum represents the nuchal ligament.[5]

Fetal cystic hygroma colli requires prenatal diagnosis for proper management. The objective of this report is to present the characteristics of the ultrasound findings and neonates.

Materials and Methods

Ultrasonographic examinations were performed by the authors from June 1989 to July 1995, using convex transducers (Aloka Model 650 or 680). Indications for ultrasonographic examinations included abnormal growth, threatened abortion, suspicion of fetal death, etc. The most important sonographic findings were cystic masses at the posterolateral aspects of the neck and the absence of spinal dysraphism or calvarial defect. Other associated anomalies were also carefully identified and documented.

When cystic hygroma was diagnosed, previous obstetric history was carefully reviewed and the counseling was given. The patient was followed until discharged from clinic.

Table 1. Baseline characteristics of the patients

No.	Age (years)	Parity	Gestation at diagnosis (weeks)	Indications for ultrasound examinations
1	30	0-0-0-0	15	Threatened abortion
2	32	0-0-0-0	16	Threatened abortion
3	24	2-0-0-2	19	Suspected fetal death
4	19	1-1-0-1	11	Threatened abortion
5	25	1-0-2-1	18	Small-for-date
6	24	0-0-0-0	12	Threatened abortion
7	36	0-1-1-1	16	Amniocentesis for genetic study
8	35	3-0-1-3	15	Large-for-date
9	20	0-0-0-0	20	Uncertain date
10	23	0-0-0-0	18	Threatened abortion
11	24	0-0-0-0	19	Large-for-date
12	38	1-0-0-1	16	Small-for-date

Table 2. Ultrasound findings and chromosome studies

No.	Ultrasound findings Cystic Hygroma	Associated Findings	Chromosome Studies
1	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck extending to axilla and mediastinum	-oligohydramnios -hydrops fetalis	Not done
2	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck	-	46, XY
3	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck extending to axilla and mediastinum	-oligohydramnios	45, XO
4	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck	-hydrops fetalis	46, XX
5	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck extending to axilla and mediastinum	-oligohydramnios -hydrops fetalis	Not done
6	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck	-	Not done
7	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck extending to axilla and mediastinum	-oligohydramnios -hydrops fetalis	47, XX ; + 21
8	-two locculations thick-walled -midline septation at posterior, posterolateral aspects of neck	-polyhydramnios -hydrops fetalis	46, XY

No.	Ultrasound findings Cystic Hygroma	Findings Associated	Chromosome Studies
9	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck extending to axilla and mediastinum	-oligohydramnios -hydronephrosis	Not done
10	-asymmetric multiseptate thin-walled -midline septation at posterior, -posterolateral aspects of neck	-	46, XX
11	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck	-oligohydramnios -marked hydrops fetalis -ventriculomegaly	45, XO
12	-asymmetric multiseptate thin-walled -midline septation at posterior, posterolateral aspects of neck	-marked hydrops fetalis	Not done

Results

Twelve cases of cystic hygroma colli were diagnosed and followed by the authors. The diagnosis of all cases was postnatally confirmed by autopsy. The demographic informations and detailed ultrasound findings are presented in Table 1 and 2 respectively. The majority of cases were complicated with threatened abortion (5 cases). Two patients, however, presented as large for date (one had polyhydramnios and the other with severe hydrops).

The maternal age ranged from 19 to 38 years, the mean age was 27.50 ± 6.45 years, 50% of the patients were primigravida.

All of them manifests as unexpected findings on the antenatal ultrasound with various indications during first half of pregnancies. The mean gestational age at time of diagnosis is 16.25 ± 2.77 weeks, range 11-20 weeks.

All of twelve cases were sonographically diagnosed in the first half of their pregnancies with no false positive. A dense midline septum could be identified in all cases. The majority of cases showed asymmetric, thin-walled, multiseptate cystic masses at posterolateral aspects of the neck. In 5 cases, cystic hygroma extended into the axilla or mediastinum. Of 12 cases, 7 had already developed some degree of hydrops fetalis (2 cases were severe). Fifty-eight percent (7 in 12 cases) had oligohydramnios and only one case had polyhydramnios. All of them had undergone elective termination of pregnancy and autopsies were performed. Three of 7 fetuses which chromosome studies were done had chromosomal abnormalities, 45 XO in 2 cases and trisomy 21 in 1 case. Excluding hydrops fetalis, other

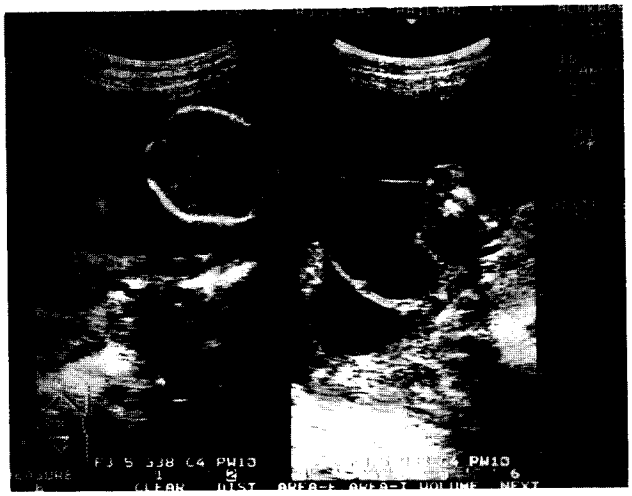

Fig. 1. Left : Cross section of the skull at the level of lateral ventricles shows ventriculomegaly and marked scalp edema.
Right : Cross section of the neck shows multisaptate thin-walled cystic hygroma.

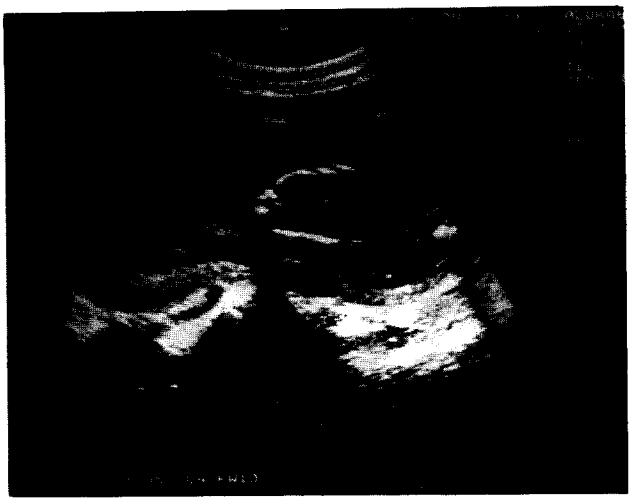

Fig. 2. Multiseptate thin-walled cystic hygroma at the posterior aspects of the neck (case 2).

Fig. 3. Cross section of fetal abdomen shows multiseptate cystic hygroma extends to the level of abdomen (A = ascites, sp = spine, c = cystic hygroma).

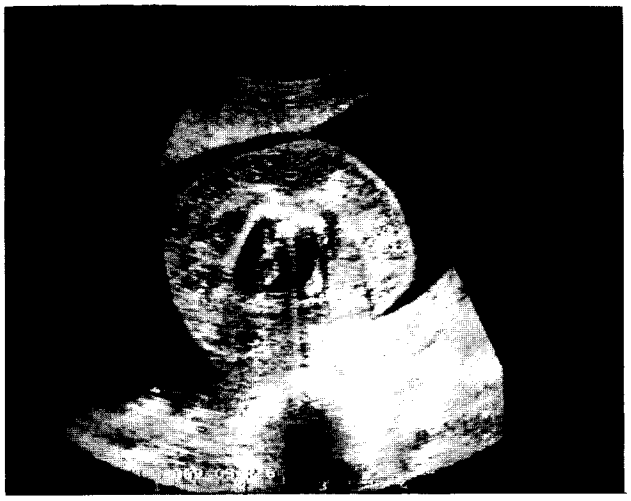

Fig. 4. Cross section of fetal chest shows marked subcutaneous edema of the fetus (hydrops fetalis).

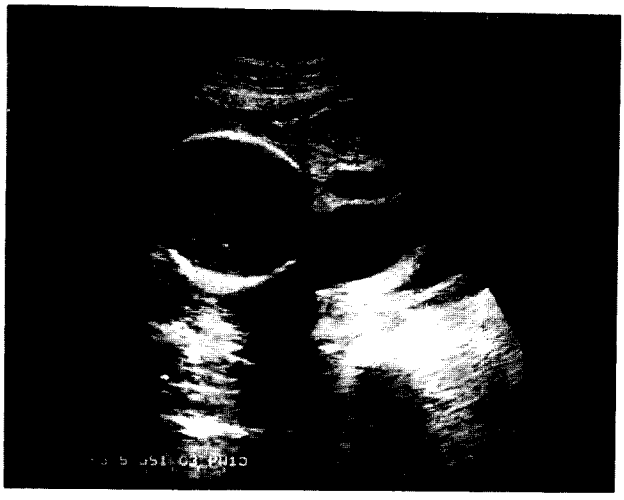

Fig. 5. Cross section of the skull shows cystic mass with thick wall at the posterior aspect of the skull (case 8).

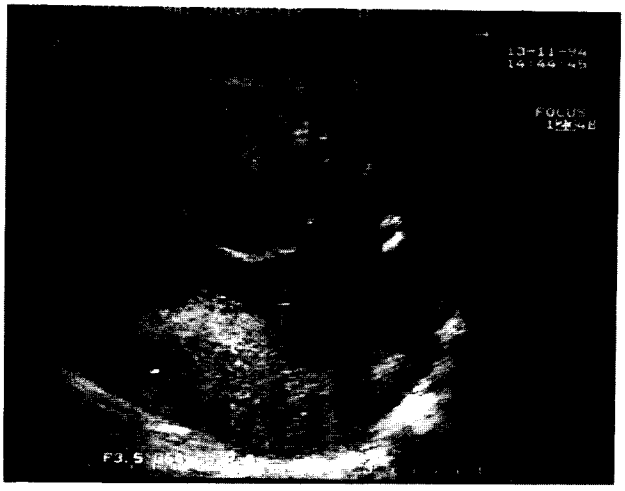

Fig. 6. Cross section of fetal abdomen shows marked ascites (case 10).

detectable anomalies included hydronephrosis (1 case) and ventriculomegaly (1 case).

Discussion

The sonographic findings of 12 cases of cystic hygroma in this study include asymmetric, thin-walled multiseptate, cystic masses without solid components at the posterolateral aspects of the neck. Interestingly, thick-walled cyst was found in one case (case no. 8 ; figure 5) and the cyst in this case had only two loculations and similar to cervical or low occipital meningocele. However, identification of the characteristic nuchal ligament and the absence of dysraphism or calvarial defect discriminates cystic hygroma from meningocele.

Similar to other reports,[3-6] oligohydramnios complicates 60% of cases whereas polyhydramnios was found in only one case. Although polyhydramnios may occur in cystic hygroma, the sonologist must not misdiagnose large fluid-filled loculations of cystic hygroma for polyhydramnios. The aetiology for oligohydramnios remains conjectural but may possibly result from fetal hypoperfusion leading to decreased renal output ; the polyhydramnios probably represents a manifestation of hydrops rather than esophageal compression. Given the common association between cystic hygroma and hydrops, the discordantly low incidence of polyhydramnios and high incidence of oligohydramnios suggests that factors leading to oligohydramnios predominate in the vast majority of cases.

Excluding hydrops fetalis and aberrations of amniotic fluid volume, other detectable anomalies associated with cystic hygroma were uncommon. We found only one case of hydronephrosis and and another case of mild ventriculomegaly.

Similar to other report,[6] midline septation extending from the posterior neck representing the nuchal ligament outlined by bilateral cysts were identified in all cases and may be the most specific sign of cystic hygroma.

Unfortunately, chromosomal study was carried out in 7 cases in this series, and only 3 cases or 43% were found to be abnormal. This is somewhat lower than in other reported series

(47-65%).[7-9] However, our series is too small to make a definite conclusion. Monosomy X was the most frequently reported karyotypic abnormality associated with fetal cystic hygroma, but trisomy also often encountered with cystic hygroma.

Because the majority of reported cases had undergone elective termination of pregnancy, the mortality rate of cystic hygroma diagnosed prenatally can not be stated with certainty. However, a compilation of small series and case reports of fetuses not electively terminated suggests a spontaneous mortalitiy rate of approximately 80-90%. The presence of hydrops fetalis or lymphangiectasia in cystic hygroma portends a grave prognosis, death usually occurring at several weeks from the time of diagnosis.[5]

Once a cystic hygroma is detected, a careful search is made for associated skin edema, ascites, and pleural or pericardial effusions. The outcome of fetuses with cystic hygromas varies but can result in intrauterine demise or partial regression, leaving a webbed neck. Rarely, they may be localized with fairly normal outcome.

In conclusion, this small series indicates that ultrasound examination of cystic hygroma typically shows asymmetric, thin-walled, multisepate, cystic masses at the posterolateral aspect of the neck but may extend into the axilla or mediastinum. Ultrasound had high predictive value in diagnosis of cystic hygroma. The common associated abnormalities were hydrops fetalis and oligohydramnios.

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REFRESHER COURSE

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Further details can be obtained from :

Dr. Kobchitt Limpaphayom

Department of Obstetrics and Gynaecology

Faculty of Medicine, Chulalongkorn University

Chulalongkorn Hospital, Bangkok 10330, Thailand

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OBSTETRICS

Medico-demographic Features of Pregnant Women with HIV Infection

Pannee Sirivatanapa MD,*
Patilak Yauwaparksopon Bs, Ed.** (Nursing)

* *Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University,*

** *OB-GYN Nursing Section, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand*

ABSTRACT

Objective To assess medical and demographic characteristics of HIV infected pregnant women attending obstetric care.

Design Retrospective descriptive study.

Setting Department of Obstetrics and Gynaecology, Chiang Mai University.

Subjetcs Between January 1989 and December 1994, 195 HIV infected pregnant women were included in the study.

Results Mean maternal age was 25.1 years, 62.1% were primigravida and 16.9% had previous history of abortion. Employee and commercial sex worker accounted for 48.2% and 2% of the cases and 6.7% had previous history of commercial sex workers. Mean age at first marriage was 21.1 years. The indications for blood testing included HIV-positive sexual partners, condyloma acuminata infection, positive VDRL, pyrexia of unknown origin and having a previous child with AIDS. Most of the cases were asymptomatic HIV infection, but 52.3% had complications during various periods of pregnancy. Deliveries with viable fetus occurred in 56.6% with mean birthweight of 2,883.7 gm, while 30.3% pregnancies were terminated with therapeutic abortion. Tubal resection and hormonal implantation were performed in 45.3% and 26.3% respectively. The prevalence of serodiscordance occurred in 9.2% of the test. Anti-HIV testing during pregnancy should always be considered with pre-and post-test counseling since this process will allow them to make a proper and correct decision with regards to children, their family life. Prevention of disease spreading and proper medical care are also the benefit of the test.

Key words : medico-demographic features, pregnancy, HIV infection

The first case of AIDS was reported in Thailand in September 1984. The HIV infected patients are now around 600,000-800,000 cases. The number of cases has been rising rapidly. The Ministry of Public Health had initiated policy of sentinel HIV serosurveillance among 7 target groups in June 1989. Pregnant population, one of 7 target groups, was found to have an increasing seropositive rate from 0 % on the survey in June 1989 to 1.5 % on the survey in December 1993. Chiang Mai, one of the large provinces in the northern part of Thailand, is reported to have the highest number of AIDS cases. Sentinel HIV seropositive surveillance among pregnant women in Chiang Mai increased rapidly from 1% on the survey in June 1989 to 7.9 % on the survey in December 1993.[1] This trend brought with it many problems in socio-economic, psychological and medical aspects. Seropositive pregnant women should be given effective counseling to reduce their anxiety about pregnancy, family life and progression of disease. The effective counseling included not only the information on the natural history of HIV infection but also the option of therapeutic abortion or continuing pregnancy and methods of contraception.

The objective of this study is to determine the medical and demographic features of pregnant women infected with HIV.

Materials and Methods

One hundred and ninety-five pregnant women with HIV infection, who attended obstetric care at Chiang Mai University Hospital during 1st January 1989 to 31st December 1994, were studied. Data of medical and demographic characteristics were collected and analyzed by percentage.

Results

Demographic features

During six years of the study (1989-1994), the total deliveries in our hospital were 42,080 and 195 pregnant women with HIV infection were found. The mean maternal age of 195 pregnant women was 25.1 ± 5.6 years (17-44 yrs). Majority of the cases were in the age group of 20-24 years (43.1%) (Table 1). Nearly half of the cases (48.2%) were employees and 2.0% of the cases still performed commercial sex working during pregnancy (Table 2). History of ex-commercial sex workers could be taken from 13 of 195 cases (6.7 %). Most of the cases (75.4%) resided in Chiang Mai, 22% lived in other 6 provinces of northern part of the country. The rest were from other provinces (Table 3). Regarding marriage, the average age at first marriage was 21.1 ± 4.5 years (13-39 yrs) (Table 4) and 50.2% of the cases had only one marriage. The maximum number of marriage was three and about 3.1% had number of six. (Table 5)

Medical features

Of 195 pregnant women with HIV infection, 121 cases (62.1%) were primigravida and 33 cases (16.9%) had history of abortion (Table 6). HIV infection before and during pregnancy were

Table 1. Maternal age (n = 195)

years	cases	percent
15 - 19	20	10.3
20 - 24	84	43.1
25 - 29	52	26.7
30 - 34	25	12.8
35 - 39	9	4.6
40 - 44	4	2.0
Unknown	1	0.5

Table 2. Maternal occupation (n = 195)

	cases	percent
Employees	94	48.2
Housewives	37	19.0
Farmers	37	19.0
Traders	20	10.3
Prostitutes	4	2.0
Unknown	3	1.5

Table 3. Residence (n = 195)

	cases	percent
Chiang Mai	147	75.4
Lamphun	24	12.3
Chiang Rai	9	4.6
Lampang	4	2.1
Phayao	3	1.5
Mae Hong Son	2	1.0
Phrae	1	0.5
Others	4	2.1
Unknown	1	0.5

Table 4. Age at first marriage (n = 195)

years	cases	percent
10 - 14	2	1.0
15 - 19	49	25.1
20 - 24	52	26.7
25 - 29	9	4.6
30 - 34	5	2.6
35 - 39	3	1.5
Unknown	75	38.5

Table 5. Number of marriages (n = 195)

	cases	percent
1	98	50.2
2	37	19.0
3	6	3.1
unknown	54	27.7

Table 6. Order of pregnancy and number of abortion (n = 195)

Order of Pregnancy	cases	percent	Number of abortion	cases	percent
1st	121	62.1	0	157	80.5
2nd	43	22.1	1	26	13.3
3rd	17	8.6	2	4	2.1
4th	6	3.1	3	2	1.0
5th	1	0.5	4	0	0
6th	2	1.0	5	1	0.5
Unknown	5	2.6	Unknown	5	2.6

Table 7. Reasons for serological testing (n = 195)

	cases	percent
ANC screening	52	26.7
Seropositive sexual partners	27	13.8
C.acuminata	23	11.8
+ve VDRL	20	10.3
Fever	7	3.6
Siblings with AIDS	7	3.6
Self request	7	3.6
AIDS	8	4.2
Herpes simplex genitalis	3	1.5
Prostitutes	3	1.5
Accidental exposure	3	1.5
History of +ve VDRL and prostitute	3	1.5
C.acuminata and +ve VDRL	2	1.0
Abnormal pap smear	2	1.0
Salmonella choleraesuis septicemia	2	1.0
Pre-medical or surgical care	12	6.3
* Others	8	4.1
Unknown	6	3.1

* M. contagiosum, IVDU, employer's request, septic criminal abortion, history of PID, recurrent vaginal candidiasis, mental retardation.

68.2% and 15.4% respectively. The reasons for serological testing as shown in Table 7 were mainly ANC screening (26.7%). The next three reasons were seropositive sexual partners, Condyloma acuminata infection and +ve VDRL. 3.6 % (7/195) had blood testing because of self request whilst 1.5% (3/195) had blood test because of accidental exposure, one from needle prick during episiotomy repair and two from conjunctival contamination of blood. There were 8 cases of AIDS, which were the reason for testing. However, there were some pregnant women who requested HIV test for themselves without any indications and a large proportion of the cases were referred to our unit because of positive blood test. Pre-medical or surgical care as the reason for testing meant that serological testing was requested by doctors, surgeons or orthopedists before performing medical care or operation.

Regarding pregnancy complications, 52.3% of cases had complications during various periods of pregnancy. Most of the cases (34.4%) had complications during pregnancy and then during pregnancy and post partum (8.7%). The main complication was infection such as condyloma acuminata, syphilis, herpes zoster, herpes simplex. Outcomes of pregnancy ended with

Table 8. Symptomatic HIV infection and AIDS cases versus manifestations of disease and outcomes of pregnancy (n = 26)

Year	No.	Antepartum	Intrapartum	Postpartum	Outcome
1991	1.	Enlarged cervical node Hepatosplenomegaly	-	-	S. abortion
1992	2.	-	-	Herpes zoster	F 2,850 gm
	3.	S. choleraesuis septicemia	-	-	M 3,700 gm
	4.	Herpes zoster	-	-	F 3,300 gm
	5.	Herpes zoster			M 3,150 gm
1993	6.	Herpes zoster	-	-	M 3,100 gm
	7.	-	-	S. choleraesuis septicemia	M 3,100 gm
	8.	PCP, oral thrush	-	-	S. abortion
	9.	CMV retinitis	-	-	T. abortion
	10.	PCP, oral thrush	-	-	M 1,240 gm
	11.	Herpes zoster, oral thrush	-	-	M 3,700 gm
	12.	-	Herpes simplex labialis	-	M 2,600 gm
1994	13.	Herpes zoster	-	-	F 2,750 gm
	14.	Herpes zoster	-	-	M 3,150 gm
	15.	Herpes zoster	-	-	T. abortion
	16.	-	-	S. choleraesuis septicemia	F 3,250 gm
	17.	PCP, oral thrush	-	Cryptomenigitis	T. abortion
	18.	PCP, oral thrush	-	-	M 2,350 gm
	19.	Chicken pox	-	Fever, Drug allergy	F 3,000 gm
	20.	Recurrent vg. candidiasis	-	-	M 3,370 gm
	21.	Recurrent fungal infection of skin	-	-	T. abortion
	22.	Chronic diarrhea, wt.loss, pruritic dermatitis	-	-	T. abortion
	23.	PCP, oral thrush, epitaxis	-	-	F 2,100 gm
	24.	-	2° ITP	-	F 2,600 gm
	25.	Herpes zoster	-	-	F 2,900 gm
	26.	Penicillosis of skin oral thrush, fungal septicemia	-	-	T. abortion

Notes : M - Male, F - Female, S.abortion - Spontaneous abortion, T.abortion - Therapeutic abortion, PCP - Pneumocystis carinii pneumonia

Table 9. No. of marriages versus result of sexual partner's serology

No. of marriage Result	1	2	3	Unknown
Negative	6	8	0	0
Positive	74	20	4	30
Not tested	18	9	2	24
Total	98	37	6	54

deliveries in 56.6%, therapeutic abortion in 30.3%. Mean livebirth weight was 2,883.7 ± 491.0 gm (range 1,200-3,940 gm). Concerning methods of contraception, tubal resection and hormonal implantation were performed in 45.3% and 26.3% of cases respectively. Six of 190 cases were unwilling to use any methods. Table 8 showed the symptomatic HIV infection and AIDS cases versus manifestations of disease and outcomes of pregnancy. Among 141 sexual partners who had blood taken for serological testing, 9.2% (13/141) had negative result. Considering the number of marriages versus the result of sexual partners's serology, we found that 6 of 98 males (6.1%) and 8 of 37 males (21.6%) whose wives got married for the first time and second time respectively still had negative result (Table 9).

Discussion

This study revealed that the majority of pregnant women were infected by heterosexual contact with their sexual partners and the age at first marriage was so young. This would result in long period of child bearing period, leading to an increase of HIV infected infants. The magnitude of HIV infected infants may be reduced through advanced planning and effective counseling to help seropositive pregnant woman make their reproductive decisions, family life-style and cope with consequences of HIV infection.

Almost half the studied cases had complications during pregnancy. The complications were coinfection with STDs and advanced stage of HIV infection affecting the outcome of pregnancy. The infants born to infected mothers with antenatal complications have a higher risk to be HIV-infanted infants. The risk of maternal-infant HIV transmission can be reduced by using zidovudine which its effectiveness maximized in the infants born to asymptomatic pregnant woman.[2,3] Though further clinical trials to evaluate the efficacy of antiretroviral therapy in symptomatic HIV infected pregnant women to reduce perinatal transmission of HIV are needed, the regimen should be presented and discussed with the pregnant women.

Our studied group belongs to the low social class for whom information is not available. Existing data shows no evidence to support the effect of HIV infection on intrauterine growth retardation. In this study, minority of infants born to infected mothers were classified as low birthweight which may be the effect of HIV infection on birthweight. The obstetricians should provide the proper antenatal care for HIV infected pregnant women to reduce the maternal morbidity and maternal-infant HIV transmission. The proper

prenatal care includes investigation and treatment fo coinfection with STDs and opportunistic infections, surveillance of intrauterine fetal growth and maternal mental support. Therapeutic abortion was the mode of pregnancy termination choosen by 30% of studied group. This figure is expected to be higher in the future. Regarding testing of HIV infected among sexual partners of pregnant women, we recommend it to be the "must" policy because 10% of sexual partners of our studied cases were seronegative. The result of testing is beneficial to themselves in changing sexual practice, family life and self care. The health care providers also have to stress to the couple on using the condom for controlling the disease spreading and slowing the advanced stage.

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Royal Thai College of Obstetricians and Gynaecologists

RESEARCH METHODOLOGY

MAY 12-14, 1997

SIRIRAJ HOSPITAL

BANGKOK, THAILAND

Further details can be obtained from :

Dr. Kobchitt Limpaphayom

Department of Obstetrics and Gynaecology

Faculty of Medicine, Chulalongkorn University

Chulalongkorn Hospital, Bangkok 10330, Thailand

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OBSTETRICS

Factors Associated with HIV-1 Infection among Adolescent Pregnancy

Surasak Taneepanichskul MD, MPH,
Winit Phuapradit MD, MPH,
Kamheang Chaturachinda MB, ChB, FRCOG.

Department of Obstetrics and Gynaecology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

ABSTRACT

Objective To study the factors associated with HIV-1 infected adolescent pregnancy.
Design Case-control study.
Setting Department of Obstetrics and Gynaecology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University.
Subjects Thirty-two cases of HIV-1 infected adolescent pregnancy and 64 cases of adolescent pregnancy with HIV-1 seronegative.
Results The mean age of seropositive adolescent was 18.58 ± 1.48 years and seronegative was 18.51 ± 1.52 years. The risk factors of HIV-1 infection among adolescent pregnancy were education less than 6 years, no contraceptive use prior to this pregnancy and syphilis.
Conclusion This study revealed the risk factors for HIV-1 infection among adolescent parturients. Education, contraception and prevention of sexually transmitted disease could reduce risk of HIV infection in this population.

Key words : adolescent pregnancy, HIV-1, risk factors

An emerging public health problem among adolescents is the epidemic of human immunodeficiency virus infection. There are very few reports concerning HIV-1 infection among Thai adolescent pregnancy. Even though HIV-1 infection in pregnancy was 2% for the whole country in 1993 but HIV infection in adolescent pregnancy had no details.[1] Since January 1991, routine voluntary HIV antibody screening has been offered to women seeking prenatal care at

Ramathibodi hospital. We previously reported 1.03% prevalence of HIV-1 infection among adolescent pregnancy while prevalence of HIV-1 infection among general pregnancy was only 0.3%.[2] The prevalence of HIV-1 infected adolescent pregnancy was about 3 times higher than general HIV-1 infected pregnancy. The objective of this report was to study the factors associated with HIV-1 infected adolescent pregnancy.

Materials and Methods

Study design : This study is case-control study.

Subjects : Ramathibodi Hospital is the 800-bed university hospital in Bangkok. All parturients who underwent antenatal care had routine voluntary antenatal HIV screening after pre-test counselling. After the result of testing was known, post-test counselling was given to all pregnant women, either positive or negative HIV testing. All HIV-infected adolescent parturients subsequently underwent follow up in a High-Risk Pregnancy clinic. The information of these adolescent pregnancies were recorded in antenatal care and delivery records. For each HIV-infected adolescent, two seronegative adolescent control were randomly chosen by random digit method. For the purpose of this study, "adolescent" is defined as a patient in the age group 15 to 20 years.

Laboratory : Blood specimens were screened for HIV-1 antibody with gelatin particle agglutination technique. Specimens which were found reactive by this technique were examined again for comfirmation by enzyme-linked immunosorbent assay (ELISA) and Western Blot technique. Those who were seropositive at the first screening test were referred to a High-Risk Pregnancy clinic and another sample was drawn for repeated GPA testing. Both reactive specimens with these techniques were considered HIV-1 seropositive.

Variables : The variables of this study are composed of age, education, contraceptive use prior to pregnancy, gravida, history of abortion, body weight at first visit, haematocrit and VDRL.

Data analysis : All data were recorded onto PC microcomputer 486/DX and analysed with statistic package program CIA and microstat. The statistic values are mean, standard deviation, odd ratio and 95% confidence interval. The analytic statistics are Student's T-test, Fisher's exact test and odd ratio. Significance is expressed at the 0.05 level.

Period of study : The period of study was from January 1, 1991 to March 31, 1996, a total period of 63 months.

Results

During the study period, 2,634 cases of adolescent pregnancy registered for prenatal care. All of them agreed to have HIV testing after pre-test counselling voluntarily. The adolescent parturients who underwent HIV screening, 32 were found to have HIV infection after the confirmation test. The prevalence rate of HIV-1 infection among this adolescent group was 1.21%. All of them were asymptomatic and none had previously known history of their infectious status. A total of 64 seronegative controls were randomly chosen to compare with seropositive patients. The mean age of seropositive adolescents were 18.58 ± 1.48 years and seronegative were 18.51 ± 1.52 years. There was no significant difference between mean age of these two groups. Table 1 shows the demographic characteristics of pregnant adolescents. There were no difference of HIV infection between age below and above 18 years old as well as housewives and other occupations among adolescent

parturients. However, mothers who had education less than 6 years were more likely to be infected than mothers who had education more than 6 years (OR = 4.33, 95% CI 1.57-11.9, Fisher's exact test P < 0.05). Table 2 shows some risk factors of seropositive pregnant adolescents. The odd ratio of adolescent parturients who were primigravida, no history of abortion and haematocrit at first visit less than 30% was higher than adolescent parturients who were multigravida, previous history of abortion and haematocrit at first visit more than 30%, but the differences were not statistical significance. However, the adolescent parturients who did not use contraception prior to this pregnancy were more likely to be infected (OR = 3.57, 95% CI 1.35-9.43, Fisher's exact test P < 0.05) than adolescent parturients who used contraception. The contraceptive methods used among these adolescents were only oral contraceptive pill and condom. There were 4 cases used condom and 3 cases used oral pill in the HIV seropositive group. In the seronegative group, there were 17 cases used condom and 15 cases used oral pill. Adolescent parturients who had positive VDRL were significantly associated with HIV seropositive (OR = 5.87, 95% CI 1.64-20.9, Fisher's exact test P < 0.05). Adolescent parturients whose body weight below 50 kilograms and those > 50 kilograms had no association with HIV infection.

Discussion

Our study showed that the important risk factors for HIV-1 infection among adolescent parturients in Ramathibodi Hospital included education less than 6 years, no contraception prior to this pregnancy and positive VDRL. The highly educated adolescent parturients in this study were less likely to be infected with HIV because there were several educational programmes on AIDS prevention in secondary schools, vocational schools and colleges by Ministry of Education and Ministry of Public Health. Adolescents who were in schools and colleges might be encouraged to protect themselves from HIV infection due to impact of these programmes.[3,4] Some contraceptive methods were also protective against HIV infection.[5] The

Table 1. Selected demographic characteristics of seropositive and seronegative pregnant adolescents

Characteristics	Seropositive (n = 32)	Seronegative (n = 64)	Odd ratio	95% Confidence interval
Age (years)				
< 18	15	31	0.94	0.40 - 2.20
19-20	17	33	1	
Education (years)*				
0-6 years	26	32	4.33	1.57 - 11.90
> 6 years	6	32	1	
Occupation				
Housewives	17	35	0.94	0.40 - 2.20
Other	15	29	1	

* Significant at 95% confidence interval

Table 2. Risk factors of seropositive pregnant adolescents

Risk factors	Seropositive (n = 32)	Seronegative (n = 64)	Odd ratio	95% Confidence interval
Gravida				
1	23	37	1.86	0.75 - 4.66
> 1	9	27	1	
Abortion				
No	23	40	1.53	0.61 - 3.86
Yes	9	24	1	
Contraceptive use prior to this pregnancy*				
No	25	32	3.57	1.35 - 9.43
Yes	7	32	1	
Haematocrit at first visit				
< 30%	5	5	2.19	0.58 - 8.19
> 30%	27	59	1	
VDRL*				
Positive	9	4	5.87	1.64 - 20.9
Negative	23	60	1	
Body weight at 1st visit				
< 50 kilograms	22	34	1.94	0.79 - 4.75
> 50 kilograms	10	30	1	

* Significant at 95% confidence interval

contraceptive methods among these adolescents prior to pregnancy were condom and oral contraceptive pill. As we know, condom can prevent sexually transmitted disease as well as HIV infection.[5] However, oral pill is still a controversy whether it has any association with HIV infection.[5-7] We found that positive VDRL was a risk factor of HIV infection among these adolescent pregnancy. Several studies showed association between HIV infection and sexually transmitted disease including syphilis.[8-18]

This study has limitation due to of small sample size of seropositive cases. In addition, we were unable to describe the characteristics and behaviors of sexual partners which may be a crucial determinant of HIV infection among these adolescent parturients. We would encourage others to investigate risk factors of these adolescent sexual partners. The outcome might be valuable for AIDS control programme among these adolescents.

In summary, this study revealed the important risk factors for HIV-1 infection among adolescent parturients in Ramathibodi hospital. Education, contraception and prevention of sexually transmitted disease might decrease risk of HIV infection in this group of population.

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XII th SCIENTIFIC AND ANNUAL

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BANGKOK, THAILAND

Further details can be obtained from :

Dr. Kobchitt Limpaphayom

Department of Obstetrics and Gynaecology

Faculty of Medicine, Chulalongkorn University

Chulalongkorn Hospital, Bangkok 10330, Thailand

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OBSTETRICS

Trends in Caesarean Section Rate in Siriraj Hospital (1980-1994)

Pornpimol Ruangvutilert MD,
Vitaya Titapant MD,
Thaviponk Suvonnakote MD, FICS.

Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

ABSTRACT

Objective To determine trends in caesarean section rate in Siriraj Hospital and the impact on maternal and perinatal mortality rates.

Design A retrospective descriptive study.

Setting Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok.

Subjects Data of all obstetric patients underwent caesarean section from Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital during 1980-1994.

Results The rate of caesarean section in Siriraj Hospital rose from 6.7 per 100 deliveries in 1980 to 18.3 in 1994. The most common indication was repeated caesarean section which rose from 2.0 per 100 deliveries in 1980 to 5.8 in 1994. Dystocia was the major contributor of the increasing primary caesarean section which rose from 1.9% in 1980 to 5.4% in 1994. Elective caesarean section had recently increased strikingly from 0.6% in 1991 to 1.43% in 1994. Maternal mortality rate declined sharply from 44 per 100,000 deliveries in 1980 to 5.5 in 1984 then increased to 24 in 1988 and then declined again to 5 in 1994. Perinatal mortality rate had been approximately 10 per 1,000 births since 1980 and remained rather constant until 1994.

Conclusion Caesarean section in Siriraj Hospital tended to continuously increase. The most common indication was repeated caesarean section. Dystocia was the most common indication for primary caesarean section. Elective caesarean section was recently increasing strikingly. Maternal and perinatal mortality were not clearly affected by these increasing caesarean section rates.

Key words : trend, caesarean section, maternal mortality, perinatal mortality

There has been a continuous rise in the rate of caesarean section at an accelerated pace over the past two decades in the United States and other developed countries.[1,2] In the United States, the rate increased from 4.5% in 1965 to almost 25% in 1988.[3] Recent studies from African and Latin American countries suggested that caesarean birth rates were increasing in ways similar to those in Western countries.[4] The rapid increase in caesarean rate in the United States and other countries had been a source of concern to both obstetricians and the general public. Several articles have tried to examine reasons for the sharp rise and suggest the way to reduce this rate.[1,5] Data of the early 1990s showed signs of a levelling off in most Western countries.[6] The rate in the United States has reached a plateau and may be falling.[3,7] However, a significant decreasing rate has not been observed.

Being an issue of international public health concern, we also aimed to assess the trends of caesarean section rate in Siriraj Hospital from 1980-1994.

Materials and Methods

Data of all obstetric patients underwent caesarean section from Medical Record and Research Unit, Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok during 1980 to 1994 were collected. From these data, caesarean deliveries were grouped into 7 categories as follow : previous caesarean deliveries, dystocia, breech presentation, fetal distress, elective caesarean section, failure of induction and others. Each indication was studied in details for time trends.

Results

In 1980, 6.7 per 100 births were delivered by caesarean section. This rate rose to 18.3 per 100 births in 1994, almost three times as high as in 1980 (Fig. 1). There appeared to be a plateau from 1987 through 1991 at the rate of about 15.6 per 100 deliveries but afterwards the rate continued to rise again.

The most common indication of caesarean

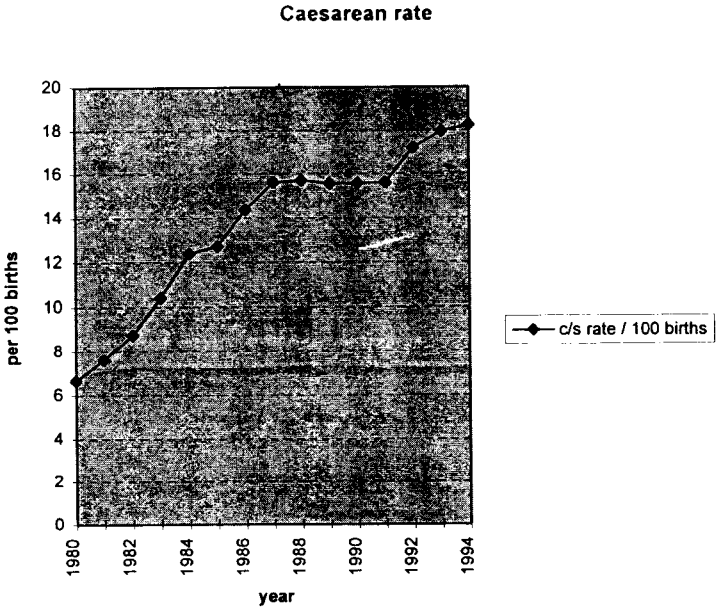

Fig. 1. Trends in caesarean section rate in Siriraj Hospital (1980-1994).

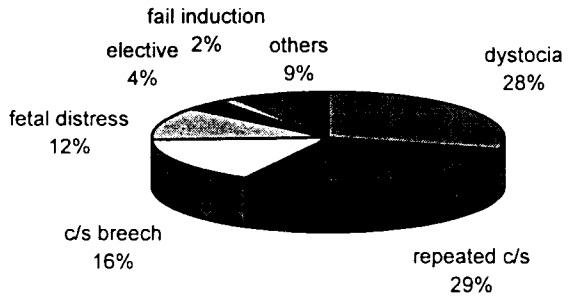

Fig. 2. Categorization of the indications for caesarean section (1980-1994).

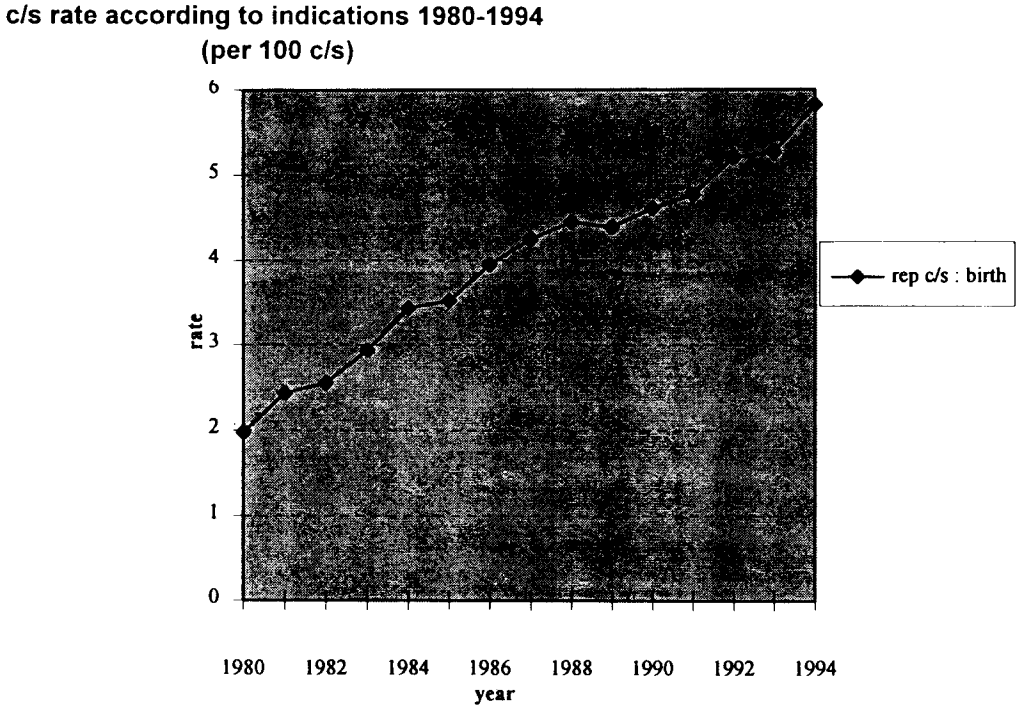

Fig. 3. Repeated caesarean section.

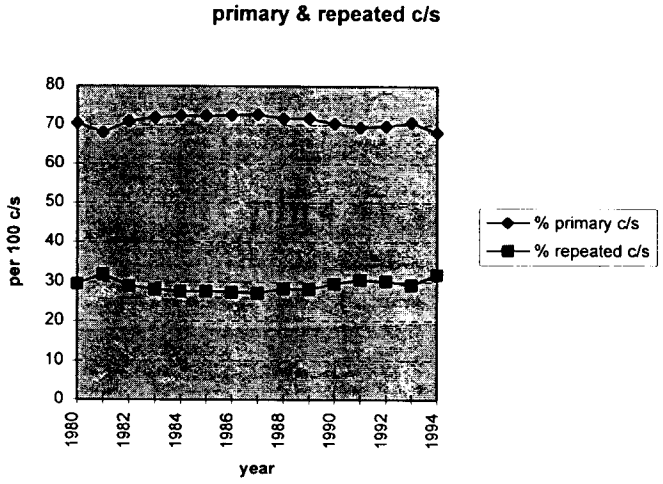

Fig. 4. Percentages of primary and repeated caesarean section.

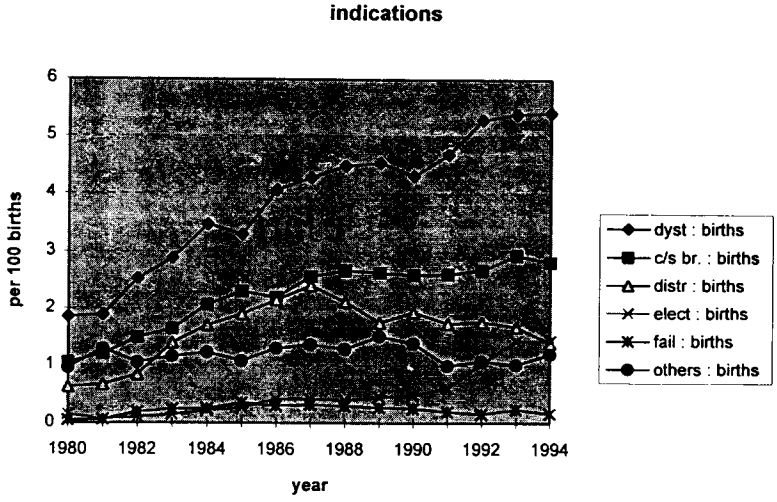

Fig. 5. All primary indications for caesarean section.

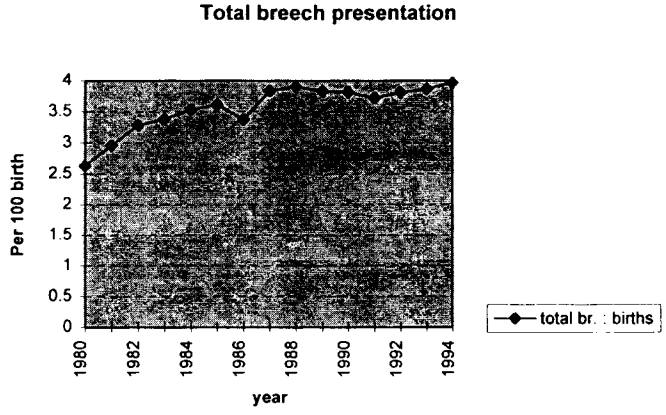

Fig. 6. Total cases of breech presentation per 100 births.

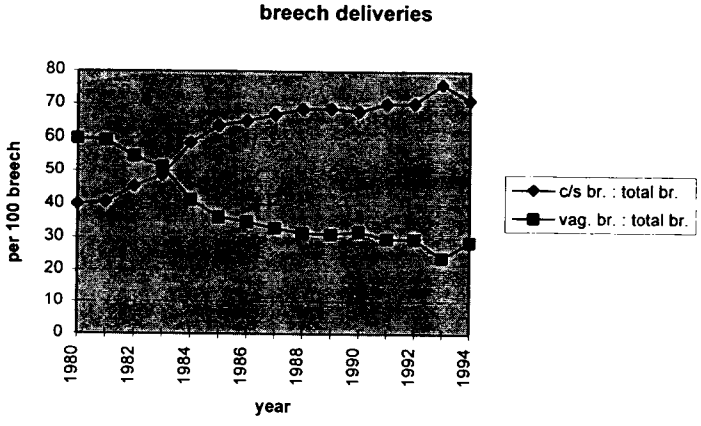

Fig. 7. Routes of delivery in breech presentation.

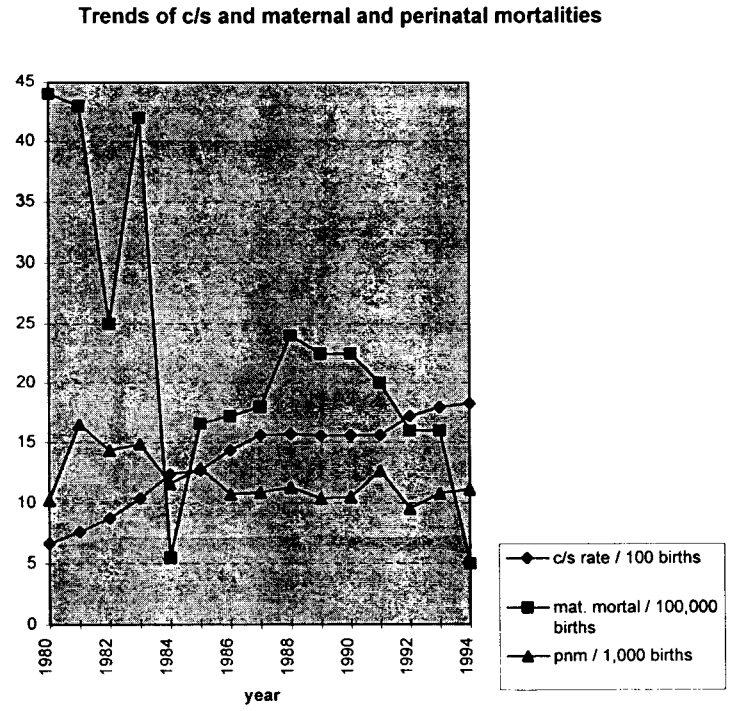

Fig. 8. Maternal mortality, perinatal mortality and caesarean section rate (1980-1994).

section was repeated caesarean section, comprising 29% of all caesarean deliveries (Fig. 2) with increasing trend throughout the studied period as shown in Fig. 3. The percentages of primary and repeated caesarean section are shown in Fig. 4 with the constant rate of repeated caesarean section per 100 total caesarean sections. From Fig. 2, the second most common indication was dystocia, comprising 28% while the third and the fourth were breech presentation and fetal distress, comprising 16% and 12% of all caesarean section respectively.

Fig. 5 shows the percentages of primary caesarean section from various indications per 100 births in 15-year period, with continuous rise of caesarean section due to dystocia as the most common indication followed by breech presentation as the second one. The recent increasing indication shown in this figure is elective caesarean section. This indication, which means performing caesarean section without trying labour and without serious indication for caesarean section, comprises of several reasons mostly for premium child such as bad obstetric history, prolonged subfertility, elderly primipara, There were no significant changes in caesarean section rate of other indications during the study period.

Concerning breech presentation, the caesarean section rate was increasing in the first few years of studied period and then it was relatively stable since 1988. Since the total number of breech deliveries was rather stable except for the slight increase in the first few years as shown in Fig. 6, therefore, the increase in caesarean section rate should be affected by the idea of changing route of delivery from vaginal to abdominal and this was confirmed by Fig. 7 which shows the route of delivery in breech presentation by time. In 1980, 40% of breech cases were delivered abdominally but since 1988, the proportion had reached 70%.

Shown in Fig. 8 are the trends in caesarean section and maternal and perinatal mortalities. As the caesarean section rate was gradually increasing year by year, trend in maternal mortality could be divided into 3 phases. In the first 5 years, maternal mortality rate declined sharply from 44 per 100,000 deliveries in 1980 to 5.5 in 1984, increased to 24 in 1988, and then declined again to 5 in 1994. Concerning the perinatal mortality, in the first 6 years, excluding 1980, the rate declined slightly and then remained virtually unchanged. These results do not support the contention that the expansion in caesarean birth rates significantly reduces perinatal and maternal mortalities.

Discussion

Caesarean section rate in Siriraj Hospital continues to rise without period of decreasing during the past 15 years (1980-1994). There was a plateau between 1987 and 1991 but afterwards the rate increased again. The various indications for caesarean section were categorized to assess the frequency of each indication and the overall 15 year-indications were shown in Fig. 2. The most common indication was repeated caesarean section with the second most being dystocia.The third, the fourth, the fifth, and the sixth were breech presentation, fetal distress, elective caesarean section and failure of induction respectively. All other indications were categorized as "others" and altogether comprised 9% of all caesarean sections. Similarly, in the USA the repeated procedures accounted for one-third of all operations and failure to progress was the most common cause in primary caesarean section.[8,9]

Concerning repeated caesarean section, the rate of caesarean section due to this indication had risen continuously during the study period. However, this increasing seemed to be passive from the increased rate of the primary caesarean section. As shown in Fig. 4, the percentage of repeated caesarean section per 100 procedures was relatively constant. Craigin's dictum "Once a caesarean, always a caesarean" is still widely practiced in Siriraj Hospital. There are several articles showing that previous caesarean section, with a selective approach, 60-80% can be delivered vaginally[10-13] and vaginal birth after caesarean section (VBAC) can steady the total caesarean section rate in the USA.[2] However, we hardly try VBAC in Siriraj Hospital so the number of total cases of previous caesarean section had been actually increasing. The indication for primary caesarean section should be more strictly respected to lessen the number of total previous caesarean section and policy of trying VBAC under certain condition should be encouraged.[14,15] However, a careful monitoring must be employed to avoid serious complications such as uterine rupture, placenta previa and varying degrees of placenta accreta.[13,16,17]

For primary caesarean section, each indication of this procedure was separately discussed.

Dystocia : The rate increased sharply from 1.9% to 5.4% (Fig. 5). The effect of active management in labour might influence this rate. With the use of Friedman curve for labour care which led us to identify abnormal patterns of labour more readily might be the main reason for this increasing rate. Midpelvic vaginal deliveries which were less likely to be attempted, was also the reason.

Breech : The total number of breech presentation had changed a little from 2.5 per 100 births in 1980 to 3.5 per 100 births in 1983 and remained rather constant thereafter (Fig. 7).

This number per se contributed very little to the increase in total caesarean rate. However, the rate of caesarean section due to breech presentation had risen from 1 per 100 births in 1980 to 2.5 per 100 births in 1987. As shown in Fig. 8, fifteen years ago, 40% of breech were delivered by caesarean sections. Since 1988, more than 70% of breech births had involved abdominal deliveries. This resulted in increasing rate of caesarean section due to breech presentation as a whole as shown in Fig. 5. Lack of practice of vaginal breech deliveries might responsible to this because some obstetricians felt that it would be safer to deliver breech cases abdominally and were reluctant to deliver breech babies vaginally. From the study of Roumen et al, a trial of labour in carefully selected patients with a child in breech presentation at term can be successfully completed in almost 80% of cases.[18] Even in primiparous term frank breech, there were no statistical differences in fetal outcomes found in the study of Wisestanakorn et al.[19] In our study, we had a plateau rate of caesarean section due to breech presentation since 1988 owing to the constant rate of total breech presentation and, more important, the constant ratio of breech presentation that were delivered abdominally. If we encourage more vaginal breech delivery we would be able to decrease the total caesarean birth rate as well.

Fetal distress : In the USA and some Western countries, the practice of defensive medicine due to rising malpractice suits has been documented and the electronic monitoring has a role in detecting fetal distress more readily than in the past and leads to an increased number of caesarean section (rationale to improvement of fetal prognosis). In Siriraj Hospital, technological monitoring was not used as widely spread as in the USA. This study showed that this indication increased until 1987 then tended to decline afterwards. So this indication contributed little to the increased caesarean section rate. So did the caesarean section due to failure of induction which, in our study, contributed very little.

Among several indications, the one which is strikingly increasing recently is elective caesarean section which means performing caesarean section without trying labour and without serious indication for caesarean section. From this study, the rate was 0.15 per 100 births in 1980 and gradually increased to 0.6 in 1991 and sharply increased to 1.43 in 1994. Elective caesarean section may be performed due to several reasons such as bad obstetric history, prolonged subfertility, elderly primipara, premium child, arbitrarily chosen time for delivery, or on patient request to avoid vaginal delivery for fear of pain or for keeping the vagina and perineum intact. Maternal age of 35 or older are frequently considered by some obstetricians to be high risk for vaginal delivery especially in nulliparous cases or cases with some medical problems which usually found in this age group.[20-24] Nowadays, older women are having children so the caesarean section rate due to elderly primipara is increasing.

Fig. 8 indicates a sharp decrease in maternal mortality rate in Siriraj Hospital from 1980 to 1983, then the rate was increasing until 1988 then it declined again. The impact of caesarean section on maternal mortality was not clear. The decreasing rate in the first 5 years might be due to the impact of caesarean section on maternal mortality if any, but the second phase of increasing rate contradicted this impact and this might be from some morbidities associated with the procedure such as infection, haemorrhage and complications of anaesthesia

especially aspiration pneumonia. Moreover, the relative risk of mortality of caesarean section compared with vaginal delivery was 5 : 1[25] and the maternal mortality after operations was 0.02-0.07%.[5] The third phase of decreasing might be the improvement in anaesthesia and surgical technique. However, the figure of maternal mortality was small and was calculated per 100,000 deliveries, only a little change in the number of cases could affect the trend and there were many other factors and causes of maternal death, Therefore the trend was not affected from the caesarean section rate alone. In other word, we could not conclude that caesarean section had impact on maternal mortality rate.

During 1981 to 1986 the increased frequency of caesarean section had been accompanied by a decrease in the perinatal mortality rate similar to what was found in the USA in the past.[5] It appeared that the increased rate of caesarean section had aided in reducing perinatal mortality. Yet a similar reduction of perinatal mortality was observed in Ireland between 1965-1980 even though the caesarean birth rate did not increase significantly, remaining between 4.1 and 4.8 percent.[26] Taylor et al found that after the elimination of major confounding factors, the substantially higher rates of caesarean section in Newark than in Dublin did not bring about a measurable reduction in the rate of neonatal losses.[27] Many other factors were likely responsible to the decrease in perinatal mortality rate, such as better prenatal care and advances in neonatal care. Moreover, there was a study suggesting that the transient pulmonary hypertension after delivery was prolonged in babies delivered by elective caesarean section,[28] and it was suggested in another study that higher functional residual capacity, higher respiratory frequency and lower tidal volume found in the caesarean section infants in that study was an adaptation to a higher pulmonary water content to ensure an efficient gas exchange with the least respiratory work.[29] In Mexico City, neonatal mortality after caesarean section was higher than vaginal birth due to preterm birth owing to uncertained date.[30] So the impact of increased caesarean section on the decreased perinatal mortality was not clear. From this study, the perinatal mortality had been unchanged despite the continuous increase of the caesarean section rate since 1987.

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Dr. Kobchitt Limpaphayom

Department of Obstetrics and Gynaecology

Faculty of Medicine, Chulalongkorn University

Chulalongkorn Hospital, Bangkok 10330, Thailand

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GYNAECOLOGY

Prevention of Rapid Trabecular Bone Loss in Women Undergoing Bilateral Oophorectomy

Nimit Taechakraichana MD,*
Kobchitt Limpaphayom MD,*
Unnop Jaisamrarn MD,*
Makumkrong Poshyachinda MD.**

* *Department of Obstetrics and Gynaecology,*

** *Department of Radiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

ABSTRACT

Objective To assess the effect of estrogen replacement therapy (ERT) on bone changes in women undergoing bilateral oophorectomy.

Design Prospective analysis.

Setting Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University Hospital.

Subjects and methods Thirty-six women undergoing bilateral oophorectomy and hysterectomy were recruited for the study. Women in the study group (19/36) received conjugated equine estrogen 0.625 mg or percutaneous 17 beta-estradiol 1.5 mg per day, while the control group (17/36) did not receive any hormone regimen.

Main outcome measures Bone mass measurement of both groups was performed at lumbar spines (L_1 - L_4) and hip (Non dominant side) by dual energy X-ray absorptiometer at 0, 6 and 12 months.

Results There was faster bone loss rate of lumbar spines in the control group than in the study group. (Percent changes of bone mineral density at 6 and 12 months, ERT : +0.45%, + 0.97%; Non-ERT : - 2.6%, -4.39% respectively, $P < 0.05$)

Conclusion This study showed the preventive effect of ERT on rapid trabecular bone loss in women undergoing surgical castration.

Key words : estrogen replacement therapy, trabecular bone loss, oophorectomized women

Osteoporosis is an increasing problem worldwide, particularly in Asia. Apart from a changing life style, increases in life expectancy enhance a substantial increase in the global burden of osteoporosis.[1]

In 1990, the average life span of a Thai female was 68 years. It is projected by the National Economic and Social Development Board of Thailand that by the year 2000, the life expectancy of a Thai female will rise to 71 years.[2] In 1993, Chompootweep et al[3] reported a study of mean menopausal age of Thai females in Bangkok using probit analysis and found that the mean age was 49 years. Hence, we can presume that a Thai female will spend at least 20 years of her life in the postmenopausal state.

Regarding osteoporosis, a large number of risk factors have been incriminated as aetiologies in which estrogen deficiency has been shown as a major factor.[4]

In natural menopause, it is the transition from regular ovulatory cycles to cessation of menstruation which is not an instantaneous event. Rather it is a series of hormonal and clinical alterations that reflect declining ovarian function.[5] In constrast to surgical menopause, after surgical oophorectomy, there is a significant reduction in sex hormone production.[6]

Many studies have associated estrogen deficiency with significant loss of bone mineral in the spine, femur, radius and metacarpal.[7-9] However, estrogen replacement therapy (ERT) has been shown to effectively protect existing bone mass in postmenopausal women.[10-12]

Hence, we conducted this prospective analysis to assess the preventive effect of estrogen replacement therapy on bone changes in oophorectomized Thai women.

Materials and Methods

Thirty-six women undergoing bilateral oophorectomy and hysterectomy and visiting menopause clinic, Chulalongkorn hospital, were recruited for the analysis. These women had no clinical bone diseases and had not received any hormone regimen within the previous one year. None were taking drugs known to affect bone changes. Women were also excluded from the study if they had evidence of chronic disease or laboratory abnormalities that could interfere with interpretation of the results of treatment. Women with an initial evaluation of bone density which revealed osteoporosis (Bone mineral density (BMD) below -2.5 SD) were excluded from the study. All participants were informed of all the details before entering the study. All patients were randomly allocated to receive estrogen (daily oral conjugated equine estrogen 0.625 mg or percutaneous 17 beta-estradiol 1.5 mg) or calcium supplement of 1,000 mg daily, with or without parasympatholytic drugs as needed.

This 12 month study was conducted in a prospective open trial method. Bone mineral density was measured using Dual energy X-ray absorptiometer (DEXA), Hologic 2000, Osteometer. Long term precision was 1.5%. A standard region of measurement, including L1-4 was scanned. Patients with severe osteoarthritic changes or compression of vertebrae were excluded from the study. Bone mineral density of hip was measured at the non-dominant side. Results are expressed in grams of ashed bone per unit area of bone scanned (gram per square centimetre, gm/cm^2). The bone measurement was performed at first visit before commencing the study and then repeated every 6 months until completing the 12 months period.

Percent changes from baseline in bone

mineral density of the lumbar spines and hip were determined after 6 and 12 months from the beginning of the study by using unpaired t-test and analysis of variance (ANOVA) where it was appropriate.

Results

Of the 36 women who completing the 12 month study, 19 women were in the ERT group (13 receiving conjugated equine estrogen, 6 receiving percutaneous 17 beta-estradiol) and 17 women were in the non-ERT group. The population characteristics of both groups are shown in Table 1. The percent changes of bone mineral density (BMD) of spine and hip in the ERT and non-ERT group are shown in Figure 1 and 2.

Discussion

It was shown that at 6 and 12 months after oophorectomy, there was a significant loss of bone mineral content of lumbar spines (L_1 - L_4) (Figure 1), the main composition of which is trabecular type.[13] However, there was no significant difference in bone changes of bone mineral content of hip within 12 months, though it showed a downward trend in the non-ERT group (Figure 2). This is probably because the hip contains more cortical bone composition than the vertebral spines. Compared with trabecular bone, cortical bone has slower bone turnover rate.[13] Nevertheless, these skeletal changes could largely be prevented by the administration of estrogen in which the study group received oral estrogen or percutaneous estrogen while the control group received no hormonal treatment. These observations are in agreement with the findings of Aitken et al[12] who showed the value of a small daily dose of mestranol in the prevention of osteoporosis after oophorectomy. Field et al[14] revealed preventive effects of transdermal 17 beta-estradiol on osteoporotic changes after surgical menopause in a 2 year study. Recently, Watts et al[6] compared the effect of oral estrogen and estrogen plus androgen on bone mineral density in surgical menopause and found that both treatment regimens prevented bone loss at the spine and hip, however, combined estrogen-androgen therapy was associated with a significant increase

Table 1. Population characteristics of the non-ERT (N = 17) and ERT (N = 19) surgical menopausal women

Characteristics	Non-ERT (Mean ± SD)	ERT (Mean ± SD)	P-value (P < 0.05)
1. Age (yr.)	46.18 ± 4.57	45.89 ± 5.86	NS
2. BW (Kg.)	62.84 ± 11.11	55.83 ± 9.65	NS
3. Height (cm.)	154.85 ± 2.93	152.84 ± 4.03	NS
4. T-men (yr.)	3.47 ± 3.09	4.68 ± 4.62	NS

ERT = Estrogen replacement therapy
SD = standard deviation, Yr = year, BW = Body weight
Kg = Kilogram, Cm = centimetre
T-men = Time since surgical menopause

in spinal bone mineral density compared with the baseline.

In this study, bone measurement was performed using dual energy X-ray absorptiometer (DEXA). This enables bone density to be measured at hip and spines with greater precision and accuracy than the other methods[15] (Single photon absorptiometer, dual photon absorptiometer, quantitative computed tomography). Apart from that, with the rapid bone loss in the non-ERT group, significant difference in trabecular bone loss of spines could be seen as early as 6 months between the study and control group.

Without estrogen replacement, the results revealed rapid bone loss (-4.39% at 12 months) in women undergoing bilateral oophorectomy (Figure 1). Christiansen's longitudinal studies of early postmenopausal women have shown that there are two characteristic groups.[16,17] Approximately 35% lost significant amounts of bone

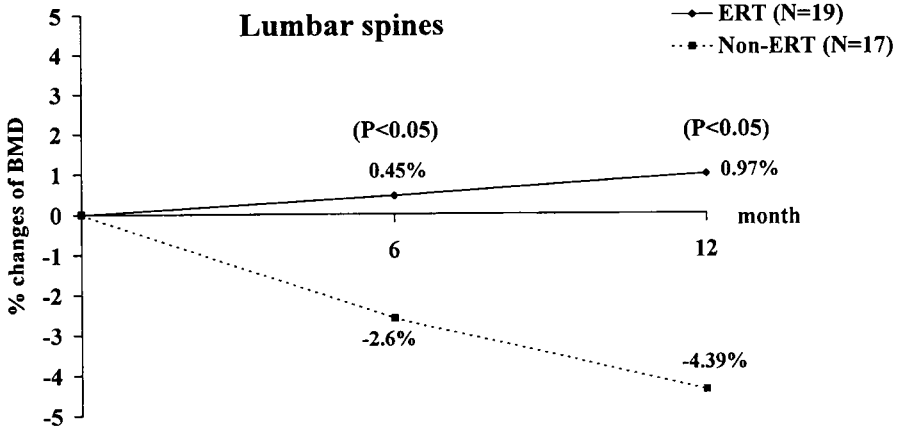

Fig. 1. Percent changes of bone mineral density (BMD) in ERT and non-ERT surgical menopausal women. (Lumbar spines : L1 - L4) (ERT = estrogen replacement therapy)

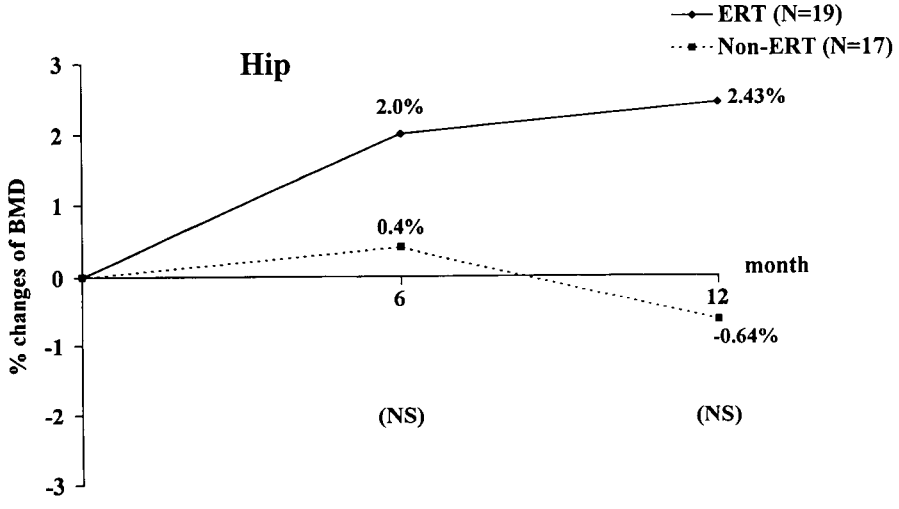

Fig. 2. Percent changes of bone mineral density (BMD) in ERT and non-ERT surgical menopausal women. (Hip) (ERT = estrogen replacement therapy, NS = No statistically significant difference)

mineral (more than 3% per year) which was classified as fast bone loser. Whereas, approximately 65% lost only a minor amount of bone mineral. Hence, women undergoing bilateral oophorectomy should also be categorized as the fast bone loser. This is because, to wait for 5 years without any prevention or treatment could result in a 15-30% loss of bone mass.[18]

In conclusion, this study showed rapid bone loss in women undergoing bilateral oophorectomy who received no estrogen replacement. Nevertheless, this could be prevented by administration of estrogen in the recommended dose as shown in this study.

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Further details can be obtained from :

Dr. Kobchitt Limpaphayom

Department of Obstetrics and Gynaecology

Faculty of Medicine, Chulalongkorn University

Chulalongkorn Hospital, Bangkok 10330, Thailand

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Paclitaxel Chemotherapy in Refractory Epithelial Ovarian Cancer : A 32 Cycle Experience

Sarikapan Wilailak MD,
Vasant Linasmita MD, FACOG,
Somkeart Srisupundit MD, FACOG,
Sunchai Bullangpoti MD,
Somsak Tangtrakul MD,
Nathapong Israngura MD.

Division of Gynecologic Oncology, Department of Obstetrics and Gynaecology, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

ABSTRACT

Objective To report our experience in using 200 mg/m^2 paclitaxel every three weeks in platinum resistant epithelial ovarian cancer patients between September 1994-August 1995.

Design Cross-sectional study.

Setting Ramathibodi Hospital.

Subjects Platinum-resistant epithelial ovarian cancer recruited for Taxol Chemotherapy between September 1994-August 1995.

Main outcome measures Response of tumour to chemotherapy according to WHO criteria.

Results The patients tolerated this regimen fairly well, acheiving one complete response, two stable disease and one progressive disease.

Conclusion Paclitaxel may be considered for refractory epithelial ovarian cancer patients with tolerable toxicity.

Key words : paclitaxel, refractory epithelial ovarian cancer

Ovarian cancer is the leading cause of gynaecologic cancer death.[1] Despite the introduction of platinum-based chemotherapy, epithelial ovarian cancer death rate remains high.[2] Seventy percent of patients with epithelial ovarian cancer will initially have a response to platinum-based chemotherapy, but resistance and progression will ultimately develop in 60% - 80%.[2,3] The

problem of treating this group of patients confronts the physicians.

Paclitaxel is a novel antineoplastic agent that is isolated from the bark of the western Yew tree, Texas brevifolia. Paclitaxel promotes microtubule assembly by preferentially binding to polymerized tubulin[4] and has documented activity against a number of solid tumours including ovarian cancer.[5]

We report our experience in using paclitaxel in platinum resistant ovarian cancer.

Materials and Methods

Patient eligibility : Patients must have (1) histologically proven epithelial ovarian cancer, (2) platinum resistance, (3) measurable disease, (4) Zubrod performance status grade 0-2, (5) expected survival of > 3 months, (6) absolute granulocyte count > 1,500/ul, platelet count > 100,000/ul, Hb > 8.5 gm/dL, (7) > 4 weeks from the last chemo/radiation therapy, (8) adequate financial support.

Treatment plan : Paclitaxel 200 mg/m^2 was administered as a 24-hour continuous infusion that was delivered in 1 L of 5% dextrose solution. All patients received standard premedication of cimetidine 300 mg and diphenhydramine 50 mg, which were given intravenously 60 minutes before paclitaxel, and dexamethasone 20 mg, which was given 14 and 7 hours before paclitaxel to prevent acute hypersensitivity reactions. Granulocyte colony-stimulation factor (Filgastim 10 ug/kg per day or Lenogastim 5 ug/kg per day) subcutaneously was initiated

Table 1. Patient and disease characteristics

Characteristic	PT. 1	PT. 2	PT. 3	PT. 4
Age (year)	36	49	41	63
Zubrod performance score	1	2	1	2
Histology	clear cell	Serous cystadeno CA	Papillary serous cystadeno CA	Serous cystadeno CA
Tumour Grade	3	2	2	3
FIGO Stage	IA	IIIC	IIIC	IIIC
Year of diagnosis	1991	1994	1992	1993
Prior chemo. regimen	- CDDP + CTX - CARBO + CTX - CARBO + CTX	- CARBO + CTX	- CDDP + CTX - CARBO + CTX	- CARBO + CTX - CARBO + CTX
Site of tumour	Pelvis	Pelvis	Pelvis	Pelvis
Tumour diameter (cm)	8	7	7.5	8
No. of paclitaxel cycles	17	3	6	6
Response	SD	PD	CR	SD
Days of G - CSF use (mean)	7.5	7.7	8.3	8.5
Mean hospital stay (day)	11.2	11.0	11.3	11.6

PT = patient, CDDP = cis - diamminedichloroplatinum, CARBO = carboplatin, CTX = cytoxan, SD = stable disease, PD = progressive disease, CR = complete response

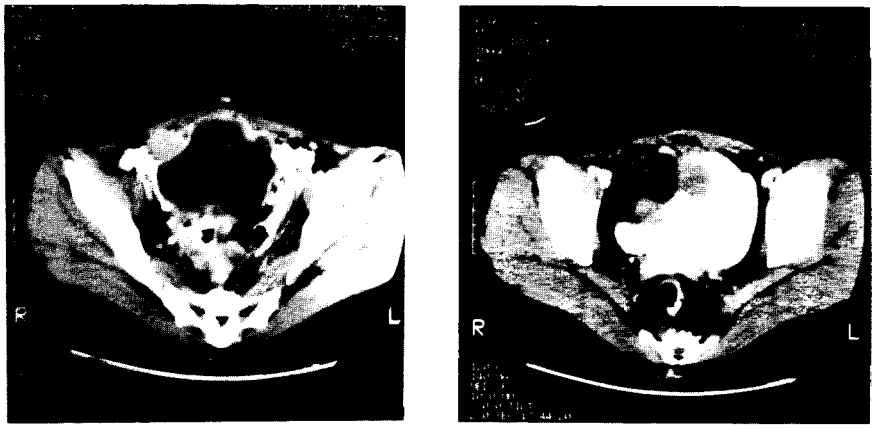
Table 2. Haematologic toxicity

Grade	PT. 1 (cycle) n = 17	PT. 2 (cycle) n = 3	PT. 3 (cycle) n = 6	PT. 4 (cycle) n = 6
WBC (x 10^3/uL)				
0 (≥ 4.0)	10	-	-	-
1 (3.0 - 3.9)	2	-	-	-
2 (2.0 -2.9)	2 (1*)	-	-	1
3 (1.0 - 1.4)	2 (2*)	3 (1*)	3	3
4 (< 1.0)	1 (1*)	-	3 (1*)	2 (1*)
Platelets (x 10^3 /uL)				
0 (≥ 100)	17	3	5	4
1 (75 - 99)	-	-	1	1
2 (50 - 74)	-	-	-	1
3 (25 - 49)	-	-	-	-
4 (< 25)	-	-	-	-
Anaemia (Hb)				
0 (≤ 11.0)	3	2	3	-
1 (9.5 - 10.5)	12	1	3	4
2 (8.0 - 9.4)	2	-	-	2
3 (6.5 - 7.9)	-	-	-	-
4 (< 6.5)	-	-	-	-

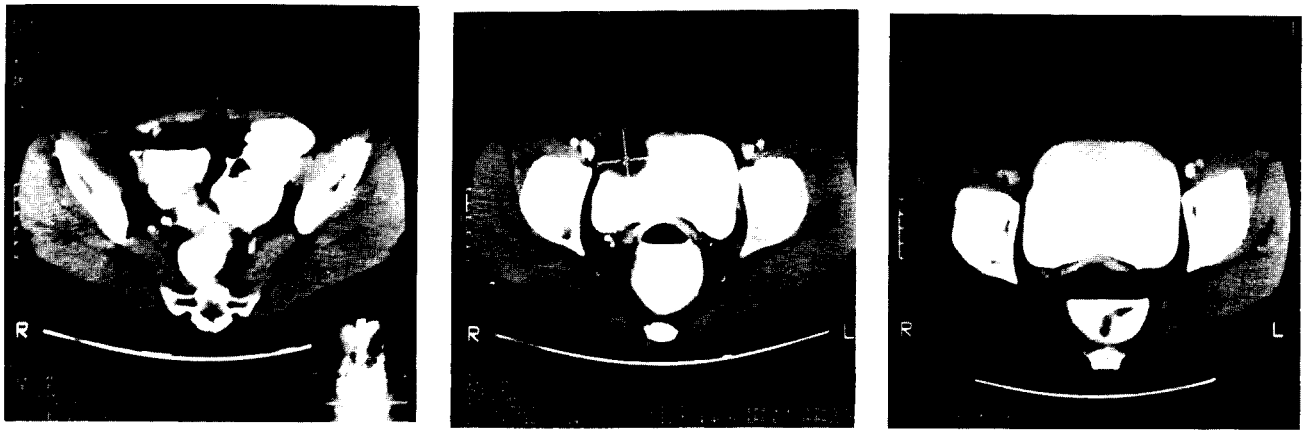
* Fever > 38°C

Table 3. Nonhaematologic toxicity

Type of toxicity	Maximum grade[9] PT. 1	PT. 2	PT. 3	PT. 4
Allergic	0	0	0	0
Gastrointestinal				
- Nausea/vomiting	1	1	1	1
- Diarrhea	0	0	0	0
Neurologic				
- Peripheral neuropathy	1	1	1	1
Cardiac	0	0	0	0
Alopecia	3	3	3	3



A, B. Before paclitaxel treatment.



C, D. After 3 cycles of paclitaxel.

E. After 4 cycles of paclitaxel.

Fig. 1. Computerized tomography of the disease in patient no. 3.

24 hours after the completion of paclitaxel until total WBC count > 3,000/ul.[6] Then the patient was discharged from the hospital. Paclitaxel administration will be repeated every 3 weeks.

Definitions : Complete response was defined as the complete resolution of disease by physical examination and radiographic examination and normalization of CA-125 lasting at least 4 weeks.

Partial response was defined as an 50% or greater reduction in the sum of the products of bidimensional measurements of all sites of disease (determined by physical examination and radiographic analysis) lasting at least 4 weeks and without the development of new lesions.

Stable disease was defined as any condition other than objective response or progressive disease.

Progressive disease was defined as a 25% or greater increase in the sum of the products of the perpendicular diameters of all measurable lesions and/or the development of new lesions.[7]

Platinum resistance was defined as disease progression during or within 6 months of the most recent platinum treatment.[8,9]

Results

During the period of September 1994 -

August 1995, there were 4 assessable patients. One case was clear cell carcinoma and 3 cases were serous cystadenocarcinoma. Patient and disease characteristics are summarized in Table 1. All had tumour recurrence/persistence in the pelvis. Patient no. 1 had stable disease after receiving 17 cycles of paclitaxel with mean G-CSF use of 7.5 days and mean hospital stay 11.2 days. Patient no. 2 had progressive disease after receiving 3 cycles of paclitaxel with mean G-CSF use of 7.7 days and mean hospital stay 11.0 days. Patient no. 3 had complete response after receiving 4 cycles of paclitaxel (after 3 cycles, tumour decreased in diameter from 7.5 cm to 3 cm and was undetectable after the 4th cycle-Fig. 1), so paclitaxel was continued for 2 cycles more. The mean G-CSF use was 8.3 days and mean hospital stay 11.3 days. Patients no. 4 had stable disease after receiving 6 cycles of paclitaxel with mean G-CSF use of 8.5 days and mean hospital stay 11.6 days.

Haematologic toxicity collected from nadir count of each series in each cycle. Haematologic toxicity was moderate and affected granulocytes more than platelets or red blood cells. Table 2 summarizes haematologic toxicity data. Febrile complication occurred in the cycles that had WBC < 3,000/uL (toxicity grade ≥ 2). Patients had fever in 7 out of 32 cycles of paclitaxel. All febrile episodes recovered after increased WBC count. Patient no. 1 and 2 had no episode of platelet < 100,000/uL. Patient no. 3 and 4 had 3 episodes of platelet < 100,000/uL. No platelet transfusion was required. No bleeding complication occurred. Mild anaemia (Hb 8-11 gm/dL) was observed in all patients.

Concerning nonhaematologic toxicity (Table 3), none had allergic reaction to paclitaxel, diarrhea or cardiac toxicity. Three patients had some nausea, patient no. 4 had some vomitting. All patients had mild peripheral neuropathy. All had complete alopecia. All treatment cycles could be restarted 21-25 days after previous cycles.

Discussion

This is our preliminary report of using paclitaxel in refractory epithelial ovarian cancer. In other reports using paclitaxel 135-175 mg/m^2, the overall response rate has ranged between 13 and 36%.[7,10-13] Using higher dose (200-250 mg/m^2), in 24 hour infusion the response rate was reported to be 48%.[6,14] So we choose paclitaxel in the dose of 200 mg/m^2 in 24 hr continuous infusion with G-CSF support with our patients. In our 4 patients with 32 cycles of paclitaxel, we obtained 1 complete response, 2 stable disease and 1 progressive disease. Report of percentage of response requires more patient number.

The patients had acceptable and managable haematologic toxicity with G-CSF support. Nonhaematologic toxicity were tolerable. The cumulative toxicity of paclitaxel was not appeared. So from our preliminary experience, paclitaxel may be considered for refractory epithelial ovarian cancer.

Acknowledgement

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REPRODUCTIVE SCIENCE

The Ultrastructural Study of the Cytoskeleton of the Human Oocytes Subjected to Micromanipulation

Panyu Panburana MD, M Med Sci in ART,*
Graham Robinson PhD,**
Simon Fishel PhD.***

* *Department of Obsterics and Gynaecology, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand,*

** *Department of Pathology, Queen's Medical Centre, Nottingham University, Nottingham, UK,*

*** *NURTURE, Queen's Medical Centre, Nottingham University, Nottingham, UK*

ABSTRACT

Objectives The objectives of the study are :- 1. To establish a technique for preparing the human oocytes for transmission electron microscopy 2. To develop an immunogold method for the localisation of cytoskeletal elements of the human oocytes 3. To study the ultrastructure of the human oocytes and the cytoskeleton, subjected to micromanipulation.

Design Experimental study.

Setting Nottingham University Research and Treatment Unit in Reproduction (NURTURE), Queen's medical centre, Nottingham University, Nottingham, UK.

Subjects and methods The oocytes, which underwent in vitro fertilization (IVF) or microassisted fertilization (MAF) e.g. Subzonal Injection of Sperms (SUZI), Direct Injection of Sperm to Cytoplasm of the Oocyte (DISCO), were collected and assigned to the control and study groups respectively at NUTURE, Queen's medical centre from 25th October to 24th December 1993.

Results The cytoskeleton (microtubules and microfilaments) has been examined by electron microscopy and immunocytochemistry. Due to their small size and number of the human oocytes, a new method for handling and preparation for transmission electron microscopy (TEM) has been developed. This method combined protein embedding with centrifugation to locate the specimens on the face of a Beem capsule mould. Therefore, it facilitated both the processing of human oocytes with minimal loss and rapid location of the specimens within the block of serial sectioning, staining and examination. The effects of microassisted (SUZI ; Subzonal Injection of Sperm, DISCO ; Direct Injection of Sperm to Cytoplasm of the Oocyte),

sucrose and sonic sword on the microfilamentous and microtubular systems of the human oocytes were studied.

Microfilaments of the human oocytes could be found at the core of microvilli and at the periphery of the cytocortex of all the groups studied by electron microscopy and electron microscopic immunocytochemistry. Most of the oocytes had a uniform microfilament distribution by light microscopic immunocytochemistry except those oocytes exposed to multiple risk factors (SUZI, sucrose, sonic sword) or the direct disturbance of the cytoskeleton system produced by DISCO.

Microtubular system of the human oocytes could be detected in all the groups by light microscopic immunocytochemistry. This showed that only two out of six oocytes from normal in vitro fertilization (IVF) (control group) and the manual microinjection of spermatozoa without sucrose and sonic sword had the normal barrel shape spindles, whilst the others had abnormal spindles. By TEM study, microtubules could be found in only one section which was cut through the chromosome and microtubule level transversely. The microtubular system could not be detected in any groups by electron microscopic immunocytochemistry. However, there was no difference in the character and distribution of other organelles in both control and study groups.

Conclusion Microassisted fertilization (SUZI, DISCO), sucrose, and sonic sword may be the risk factors of the human oocyte cytoskeleton abnormality, especially those exposed to combined risk factors or the direct disturbance of the cytoskeleton system produced by DISCO.

Key words : ultrastructure, human oocyte cytoskeleton

Although the modern era of the research in cytoskeleton is only about a decade old, the roots of the cytoskeleton concept can be traced back to the early days of the cell theory. The term "cytoskeleton" was introduced by John T. Needham more than 2,000 years ago. The cytoskeleton is the system of fibrillar structures in the cytoplasm of eukaryotic cells. The three main types of fibrils forming these structures are microtubules, microfilaments and intermediate filaments. This integrated system of molecules provides the cells with shape, internal spartial organization, motility, cell division, gamete fertilization, and possibly a means of communication with other cells and the environment.

With the advent of in vitro fertilization (IVF) and microassisted fertilization (MAF) as accepted clinical procedures in the modern reproduction, it becomes interesting to study the ultrastructure of cytoskeleton in the human oocytes and embryos in order to understand some kinds of abnormalities such as triploidy, aneuploidy and also growth differentiation of the embryo.

Materials and Methods

The stimulation protocols, (Clomid/HMG, HMG/FSH and Buserelin/HMG), were used to obtain multiple oocytes at Nottingham University Research and Treatment Unit in Reproduction (NURTURE), Department of Obstetrics and Gynaecology, Queen's Medical Centre Nottingham University from 25th October - 24th December 1993. Oocyte recovery from the follicles was achieved by a transvaginal ultrasound technique approximately 36 hours after the injection of human chorionic gonadotrophin (HCG). The

retrieved oocytes were transferred into microdrops of Earls' media containing 10% human serum under liquid paraffin oil in an atmosphere 5% CO_2 in air and maintained at 37° C for not less than 6 hours. The oocytes underwent in vitro fertilization (IVF), or microassisted fertilization (MAF) e.g. Subzonal Injection of Sperms (SUZI), Direct Injection of Sperm to Cytoplasm of the Oocyte (DISCO) (Embryology protocol manual, NURTURE, 1993). After 14-22 hours, the oocytes were examined for pronuclei formation. The mature unfertilized oocytes (aged 48 hours) were collected and assigned to the control and study groups.

Control Group :

Unfertilized nonmicroinjected human oocytes and fertilized abnormal nonmicroinjected human oocytes

Study Group :

Unfertilized microinjected human oocytes and fertilized abnormal microinjected human occytes

Group A = Subzonal injection of sperm (SUZI) group

Group A1 = Normal (manual) microinjected oocytes

Group A2 = Microinjected oocytes with sonic sword

Group A3 = Manual microinjected oocytes by using sucrose to create more perivitelline space

Group A4 = Microinjected oocytes with sonic sword by using sucrose to create more perivitelline space

Group B = Direct injection of sperm to cytoplasm of the oocyte (DISCO) group

Following examination, all the oocytes were then transferred with a heat-polished micropipette to drops of 2.5% glutaraldehyde in 0.1M phosphate buffer (GA ; pH 7.4) and fixed at 37° C for 45 minutes. The specimens were washed three times in phosphate buffer saline (PBS) and kept at 4° C for examination by electron microscopy and immunocytochemistry.

Electron Microscopic (EM) Study of the Oocytes

Five oocytes in each group were washed in 10% bovine serum albumin in Dulbecco's phosphate buffer (BSAD). Then, those in each group were transferred to a dust-free Beem capsule containing one drop of BSAD. The oocytes were left for 30 minutes to settle on the bottom of the capsule. Any specimens adhering to the sides of the capsule were gently dislodged under a dissecting microscope with the closed end of a heat-polished micropipette. When the specimens were situated on the bottom, the capsule was centrifuged horizontally for 15 minutes at 1,800g. Three drops of GA were dropped onto the surface of the BSAD and the capsule was centrifuged as described for an additional 60 minutes. The capsule was then filled with GA and refrigerated at 4°C overnight.

The following morning,GA was poured off. The capsule bisected longitudinally with a scalpel blade and the gel containing the occytes was removed. This specimen was transferred to a vial for post-fixation in 1% aqueous osmium tetroxide at room temperature for 2 hours, dehydration and infiltration. For embedding, the gel was carefully aligned in the bottom of a Beem capsule containing three drops of resin. The capsule was then filled with resin and polymerized at 60°C for 18 hours. 1 μm sections were cut with glass knives using a Reichert-Jung ultracut ultramicrotome and stained with 1% toluidine blue in borax.

Ultrathin sections (approximately 70nm) were obtained with Diatome diamond knives using LKB and Reichert ultramicrotomes. Alternate series of 1 μm and ultrathin sections were cut for routine examination. Thick sections were photographed with Leitz microscopes. Thin sections were mounted on uncoated copper grids, stained with alcoholic uranyl acetate and Reynoldís lead citrate, and examined with a Jeol JEM1200 EX electron microscope and photographed at original magnification ranging from x 5,000 to x 100,000.

Immunocytochemistry Study

Light Microscopic Level With Immunogold-Silver Staining

Two oocytes in each group were treated in 0.04% Triton X-100 in PBS for 30 minutes and then washed twice in PBS for 5 minutes. Oocytes were transferred in 50 mM ammonium chloride for 15 minutes and then washed twice again in PBS for 5 minutes. One oocyte in each group was then incubated in monoclonal mouse and alpha-actin (1 : 50) and the other was incubated in monoclonal mouse anti alpha-tubulin antisera (1 : 1000) for 60 minutes at room temperature. Following incubation, the oocytes were washed twice in PBS for 15 minutes and incubated in 5 nM immunogold conjugated goat antimouse immunoglobulin G (1gG) (1 : 50) for 30 minutes. They were then washed three times in distilled water for 3 minutes and incubated in silver enhancer for 10 minutes. The oocytes were washed again in distilled water for 3 minutes three times. Whole oocytes were mounted in Aqueous Mounting Media (Nustain). Qualitative evaluation of the morphology of the cytoskeleton was done with a Leitz microscope at a magnification of x 400. If the meiotic spindle was present, its morphology was considered normal when barrel-shaped and abnormal when reduced, elongated, or disrupted. For the methodology control, an oocyte from the control group was attained as detailed above, except that incubation in the primary antiserum was omitted. Photographs were taken on Fuji film using Leitz photographic installation.

EM-Level With Post-Embedding Immunogold Staining

Two ultrathin sections from one oocyte of each group were collected onto pre-cleaned uncoated nickel grids (200-300 mesh). Grids were put into drops of a saturated aqueous solution of sodium metaperiodate for 15 minutes at room temperature. Subsequently, the grids were washed in drops of water three times for 15 minutes and then put in drops of 1% Triton X-100 for 15 minutes. The grids were then put in drops of 1:5 swine serum for 1 hour. One grid from each oocyte was then incubated in drops of monoclonal mouse anti alpha-actin (1 : 50) and the other was incubated in monoclonal mouse and anti-tubulin (1 : 1,000) antisera overnight at 4° C. Following incubation, grids were thoroughly washed five times in 1% BSA in TRIS-buffered saline (TBS-BSA) for 15 minutes and then incubated for 60 minutes at 37°C with the immunogold conjugated goat and mouse IgG diluted 1 : 15 in TBS-BSA. After extensive washing in TBS-BSA, sections were postfixed for 10 minutes at room temperature in 2.5% glutaraldehyde in 0.1M PBS and then washed twice in distilled water for 15 minutes and allowed to dry in a dust free environment. For the methodology control, the ultrathin sections from one of the control oocytes was stained as detailed above, except that incubation in the primary antiserum was omitted. Grids were counterstained with Reynoldís lead citrate and uranyl acetate,

and examined with Jeol JEM 1200EX electron microscopes. The specimens were photographed at magnification ranging from x 5,000 to x 130,000.

Results

In order to assist in the interpretation of the results concerning changes in the cytoskeleton system of the human oocytes subjected to micro manipulation, it was considered necessary to document the basic ultrastructure of the oocytes in each group first. Therefore, the new method for handling and preparing the human oocytes for EM was done. The blocks of resin were cut into thick sections (1 μm) and stained with Toluidine blue before the ultrathin sections (approximately 70 nm) were obtained.

Toluidine blue is extremely useful when sections for EM are needed as they give a general idea of the orientation of the tissue and enable one to pinpoint areas of interest in the block face prior to further trimming of the block of ultramicrotomy. Toluidine blue stained slides will also allow the quality of fixation and embedding to be assessed. One μm resin sections of oocytes stained with toluidine blue are of considerable aid to the embryologist to assess the oocytes roughly at different levels (Fig.1).

Basic Ultrastructural Study by TEM

The human oocyte was surrounded by a fibrillar zona pellucida, outside which there were several layers of cumulus cells. The perivitelline space was found within the zona pellucida. The oolemma protruded into numerous microvilli during the early stages of maturation, which progressively decreased in number as maturation proceeded. Each microvillus had a core of microfilaments which could be found in all of control and study groups.

Microfilaments also formed a more or less continuous band beneath the oolemma of the immature oocyte. This band could be found only in the group A2 (Fig. 2).

Cortical granules(CG) were found in both control and study groups without any significant difference. They became progressively abundant as the oocyte completed maturation[1] and migrated to the surface beneath the oolemma. These granules, characteristic of all mammalian oocytes, were bound by well-defined membranes and contained extremely electron-dense cores. They were formed by hypertrophic Golgi complexes, initially, abundant in the cortical ooplasm.[2] The cortical reaction involved the exocytosis of the contents of cortical granules when the sperm fused with the oocyte at fertilization. A wave of cortical granule release is believed to be propagated circumferentially from the point of sperm entry, and the reaction is completed within a few minutes. The cortical granules burst open and their membranes fused with the overlying oolemma, releasing their contents into the perivitelline space, by a process of cell secretion (Fig. 3). The oolemma at the point of exocytosis was quite dense and appeared to be straitened (Fig. 3).

Golgi complexes were composed of elongated cisternae, vesicles, and vacuoles, there was no difference in distribution in both control and study groups.

Lysosomes existed in a variety of forms and were involved in intracellular digestion. They were acid phosphatase positive and heterogenous in overall appearance. Primary lysosomes were small vesicles bound by a limiting membrane and contained a dense core, often eccentric in position. Secondary lysosomes were larger and showed membranous and vesicular profiles associated with dense material. These were

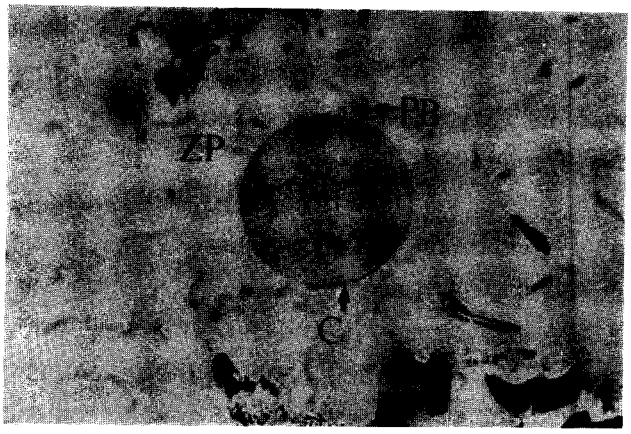


Fig. 1. The IVF mature oocyte of the control group x 400.

This light micrograph shows the zona pellucida (ZP) and one polar body (PB) in the perivitelline space. The ooplasm has an even distribution of organelles. Numerous cortical granules (CG) are organized in one discontinuous layer beneath the oolemma. The cumulus cells (CC) have been removed but there are some left.

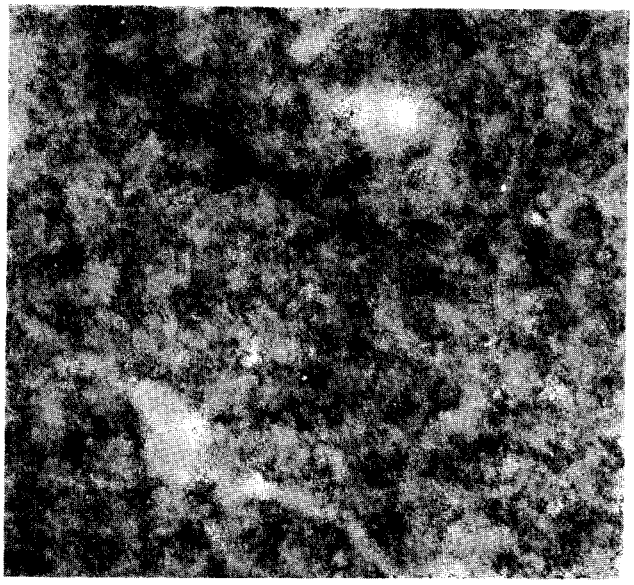


Fig. 2. The cytocortex of the human oocyte (group A2) TEM x 10,000.

This micrograph reveals the surface architecture of the junction of the polar body and the oocyte. The disrupted band of microfilaments (arrow) locates beneath the oolemma.

Fig. 3. The cortical granules (group A1) TEM x 30,000.

These cortical granules burst open and the membranes fuse with the overlying oolemma, releasing their contents into the perivitelline space. the oolemma at the point of exocytosis is quite dense and appears to be straitened.

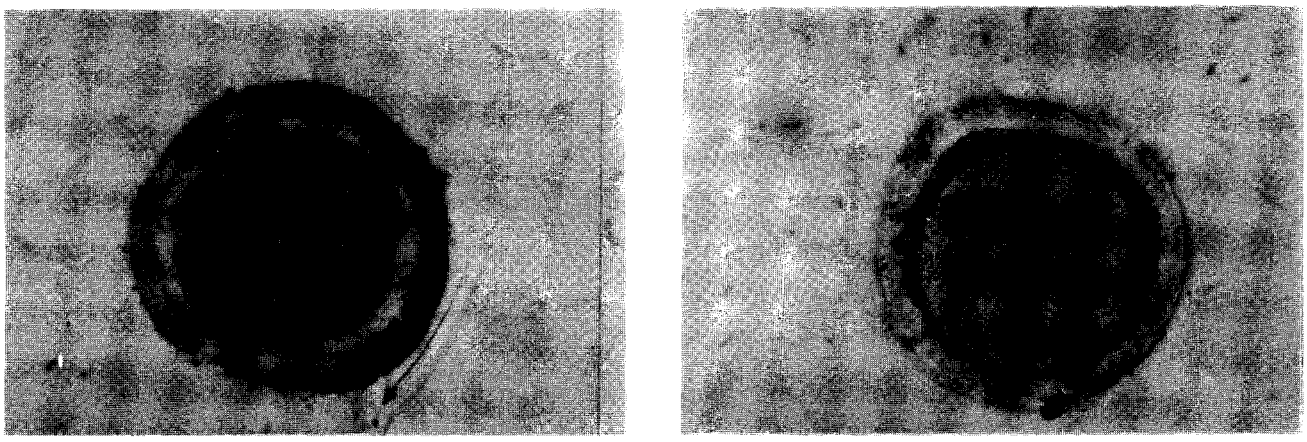
probably digestive or autophagic vacuoles, which later gave rise to residual bodies with ill-defined contents. The latter appeared to exocytose their contents at the oocyte surface. The lysosomes in both control and study groups had no difference in the character and distribution.

The endoplasmic reticulum was predominantly smooth (SER) and consisted of three types of elements: vesicular, tubular and irregular. Rough endoplasmic reticulum composed of elongated cisternae was seen only in oocytes from antral follicles, during early maturation. The predominant type of SER was vesicular and distributed randomly in both control and study groups, whereas the tubular form was rarest.

Ribosomes were generally inconspicuous and sparse in all groups. Mitochondria are oval or spherical and usually had dense stroma and

Table 1. Microtubule Distribution

Group	Normal Distribution	Abnormal Distribution
C	normal	
A1	normal	
A2		disruption
A3		disruption
A4		disruption
B		disruption



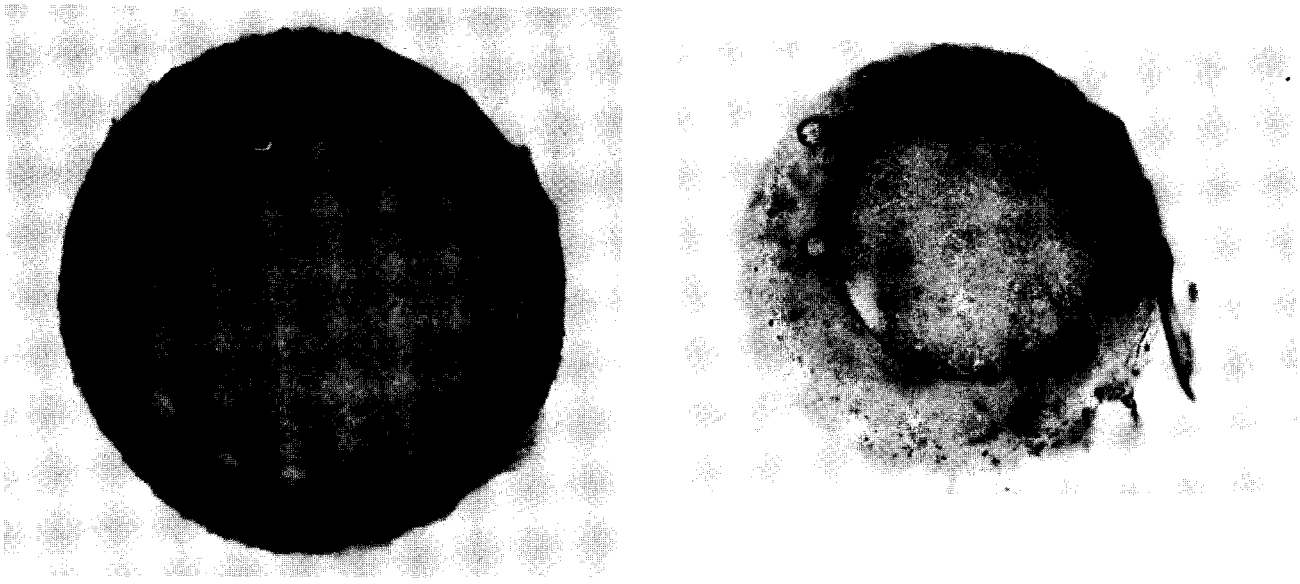
A B

Fig. 4. Light microscopic immunocytochemistry x 400.

Unfertilized human occytes show meiotic spindle visualized by immunogold-silver staining after anti-alpha tubulin labelling. The oocytes of the control group and group A1 reveal normal barrel-shaped spindles with broad anastral poles (Fig. 4a). The spindles of oocytes of of group A2, A3, A4 and B have disrupted spindles (Fig. 4b).

Table 2. Microfilament Distribution

Group	Uniform	Diminished Uniform	Non - Uniform
C	+		
A1	+		
A2	+		
A3	+		
A4		+	
B		+	



a. Control group

b. Group B.

Fig. 5. Light microscopic immunocytochemistry x400.

The unfertilized human oocytes show actin visualized by immunogold-silver staining after anti-alpha labelling. The control group and group A1, A2, A3 show uniform distribution throughout the cytocortex. But two oocytes from group A4 and B show diminished distribution.

peripheral, transverse, or reticulate cristae. Their matrixes became less electron dense while their cristae were more prominent in early embryo.[2] Mitochondria were the most conspicuous organelles and had no difference in both control and study groups.

Normally,the second meiotic division begins almost immediately after the first meiotic division. There is no interphase and nuclear formation. Prophase 2 is very short or almost nonexistent. The chromosomes align themselves on the equator of the second maturation spindle (metaphase 2), and the second arrest begins at which stage the oocyte is ovulated. Only one section cut through the chromosome level of group A1.

The first polar body contained cortical granules and isolated chromosomes associated with residual microtubules. It could easily be mistaken for a second polar body when view with phase microscopy. This would lead to a false assumption that the oocyte was fertilized, especially if the first polar body fragmented into two or three portions.

The second polar body differed from the first in that it had very few cortical granules, and its chromatin was organized into a spheroidal nucleus. There was no difference of polar bodies in both control and study groups.

Immunocytochemistry Study (Light Microscopy)

A total of twelve human oocytes were studied. Six of these (one in each group) were stained with anti-alpha tubulin by immunogold-silver enhancement technique to determine the distribution of microtubules. The other six oocytes (one in each group) were stained with anti-alpha

actin by this technique in order to determine the distribution of microfilaments.

Microtubules were detected in all the human oocytes (Table 1). Two oocytes from the control group and group A1 showed normal barrel shaped anastral spindles (Fig. 4a). The other four oocytes from group A2, A3, A4, and B revealed abnormal-shaped spindles (Fig. 4b).

Microfilaments were detected in all the human oocytes (Table 2). Four oocytes from the control group, A1, A2 and A3 groups showed uniform distribution throughout the cytocortex (Fig. 5a). But two oocytes from group A4 and B showed diminished uniform distribution (Fig. 5b).

The negative controls, in which incubation in the primary antiserum was omitted, showed no specific staining.

Immunocytochemistry Study (Electron Microscopy)

Two of the ultrathin sections from one oocyte in each group underwent post embedding immunogold staining. One ultrathin section of each group was stained with anti alpha-actin. The groups of immunogold dots could be detected at the periphery of these six oocytes (Fig. 6, 7).

The other of each group was stained with anti alpha-tubulin by this technique. Unfortunately, no chromosomes in these oocytes were sectioned and the immunogold dots found in these sections were not specific.

The negative controls, in which incubation in the primary antiserum was omitted, showed no specific staining.

Discussion

The cytoskeleton of the mammalian oocyte is essential, not only for the maintenance of chromosomal organization but also for the extrusion of the polar body and for the complex intracellular movements associated with syngamy and cleavage.[3] It has been believed that MAF(SUZI,DISCO), sucrose and sonic sword can induce spindle alterations and chromosomal anomalies.[4,5] The human oocytes from IVF and MAF were collected in order to study the effects of these procedures on the cytoskeleton by EM

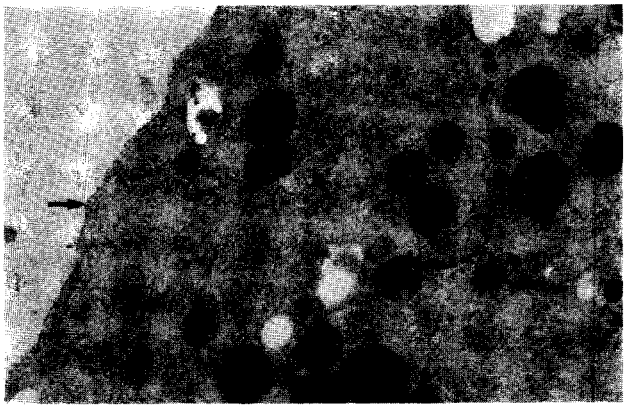


Fig. 6. Immunogold dots (control group TEM x 13,000. This micrograph shows groups of immunogold dots (arrow) along the periphery of the oocyte.

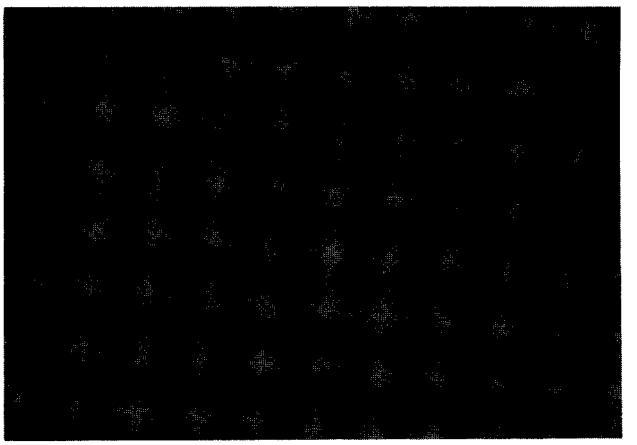


Fig. 7. Immunogold dots (control group) TEM x 130,000. High magnification of the group of immunogold dots (arrow) is shown in the periphery of the oocyte.

and immunocytochemistry.

The preparation of oocytes and embryos for light and transmission electron microscopy is complicated by the small size of the specimens and the relative ease with which they can be lost during handling. By embedding the specimens in agar, the oocytes can be routinely processed without the need for a dissecting microscope and carefully transferred from one solution to the next.[6] However, unless situated in the same plane at the surface of the agar block, locating and sectioning oocytes can be tedious and time consuming.[7] To solve these procedural difficulties, the oocytes were embedded on the face of a protein mould of a Beem capsules. This is the first application of this method to the study of the human oocytes.

Centrifugation of the oocytes and addition of the glutaraldehyde by dropping it into the capsule minimized disruption of the specimens and ensured that they would be aggregated on the surface of the mould following fixation. Shape retention was crucial for alignment of the mould in a Beem capsule for polymerization. Where care was taken to align the mould accurately in the capsule during embedding, the specimen was situated 10-130 μm below the surface of the resin block. The concentration of the specimens in a single section depended on the degree of parallelism which was attained between the knife and the face of the BSAD mould itself. Furthermore, it was critical that the dishes, micropipettes and capsules used to handle the oocytes were completely free of extraneous particles. Particles adherent to the zona pellucida occasionally cause damage to the knife during sectioning.[8]

Centrifugation of unfixed oocytes from domestic animals at forces of 10,000 g or greater leads to stratification of cytoplasmic organelle distribution was noted with the centrifugal force used in this method. This may be due to fixation prior to centrifugation and/or the ower centrifugal force required to embed the oocytes. The major advantage of this method was the reduction in time required to locate, section, stain and examine of the human oocytes.

By TEM, each microvillus has a core of microfilaments extending to the cortical ooplasm and mingles with the cortical band of microfilaments.[2] Such microfilaments are now believed to be composed of actin (probably associated with myosin) and considered to play an important role in the motility of microvilli at the cell surface.[2] In only one section of group A2, the cortical band of microfilaments could be detected beneath the oolemma. This is because this band tends to be small and disrupted in the mature oocyte. This band was first identified by Sathananthan et al (1984). It is believed to prevent the cortical granules, organized in a single layer, from migrating to the periphery at the immature stage. It disorganizes later at the germinal vesicle stage, when the granules appear to migrate to their final location, immediately beneath the oolemma. In telophase, the oocyte displays increased staining at this band in the area of the constriction furrow between the oocyte and polar body.[2] The aged human oocytes demonstrate a diminution of intensity of actin staining. Sometimes ,the bands of polymerized actin are not detected in some aged human oocytes.[3] Defects in the actin system in the oocyte are often associated with abnormal extrusion of the second polar body leading to triploidy.[3]

Microtubules were detected in the only one ultrathin section which cut through the chromosome level of group A. Microtubules could not be clearly identified because the sections were cut transversely. Normally, the oocyte is arrested at

metaphase of the second meiotic maturation, when it is ovulated. The chromosomes organize at the equator of a spindle. The spindle is more or less barrel shaped at this stage. Microtubules insert into the kinetochore (centromere) of each chromosome. The kinetochore appears as a crescent shaped dense body located in the constricted region of the chromosome. When the oocytes is activated or fertilized, the appearance of small strands or bundles of microtubules in the cytocortex can be detected and by late telophase, they become much more abundant. The organization of the meiotic spindle requires both chromosomes, which cause a local reduction in the threshold for microtubule polymerization and the pericentriolar material to nucleate microtubule polymerization. Human meiotic spindles have centrosomes but no centrioles. Centrosomes and centriole are both self-reproducing organelles and centrioles merely advertise the presence of centrosomes. In human embryos, centrioles are paternally derived.[8] Sathananthan et al (1991) demonstrated that the presence of male centrioles associated with centrosomes in the first mitotic spindle of the human fertilized oocyte.[8]

Microtubules play a central role in rapid organelle movement in animal cells in vesicle-mediated transport from Golgi to ER and in intercompartmental transport.[9] In addition, microtubules are involved in maintaining the Golgi structure since disruption of the microtubular network during mitosis or drug-induced disassembly leads to fragmentation and scattering of Golgi fragments throughout the cell.[10] Reassembly of the microtubular network results in reaggregation of the Golgi cisternae at the Microtubule Organising Centre (MTOC). During this process the Golgi elements move along microtubules; neither immediate filaments or microfilaments appear to be involved in the reassembly.[11] There are also some reports that the actin-based microfilament system is involved in intracellular vesicle transport.[12,13] However, there is no difference in the distribution of the organelles among all groups of the oocytes in this study.

In this study, the immunogold and immunogold-silver staining techniques were applied for the first time to demonstrate the cytoskeleton of the human oocyte by EM and light microscopy respectively.

With immunogold-silver staining techniques, the microtubule spindles and microfilaments could be detected in all of the oocytes by light microscopy. Only two occytes from the control group and group A1 showed normal barrel shape spindles while the other (group A2, A3, A4 and B) showed abnormal spindles. This can be explained that the occytes in group A2, A3, A4 have at least two risk factors to disturb the microtubular system ; e.g. MAF, SUZI, sucrose, and sonic sword. For the oocyte in group B, the spindle fibres may be disturbed directly from the micromanipulation (DISCO). It is possible that as the unfertilized human oocytes in this research was not fresh, the distribution and morphology of the microtubules would be abnormal. The study of Eichenlaub-Ritten (1988) revealed that the spindle of occytes aged for 48 hours was rather small and bipolar or multipolar.[14] Chromosomes were no longer aligned at the spindle equator, but were scattered all over the degenerated spindles. Four oocytes from the control group, group A1, A2, A3 have the uniform distribution of the microfilament, while those from A4 and B have diminished distribution detected by light microscopic immunocytochemistry. The reason may be that three risk factors (SUZI, sucrose and sonic sword) are present in group A4 and direct disturbance of microfilament is the risk factor of group B.

Immunogold-silver technique was compared with the FITC (fluorescein isothiocyanate-immunofluorescence) technique.[15,16] The basic principles of both techniques are similar. An immunolabelling of the spindle with a monoclonal anti-tubulin antibody is followed by an incubation with a second antibody. The difference in methods of visualization of the spindle depends on the nature of the conjugate of the second antibody, being either an FITC molecule or a gold particle. A potential disadvantage of the immunogold-silver staining technique could be the longer procedure time required compared to FITC- immunofluorescence. The obvious advantages of the immunogold-silver staining technique were the production of permanent preparation and no requirement of a special kind of microscope.

From EM immunocytochemistry study, no difference could be found among the microfilament distribution of all the occytes, while the microtubular system could not be detected in any of the groups.

The development of light and electron microscopic immunocytochemistry has provided us with a correlation between structure and function and brought a significant improvement in our knowledge of cell cytoskeleton system.

Immunogold staining was chosen in this study because it is an indirect method which is more sensitive than the direct method and providing the primary antibody host species remain the same, any number of tests can be performed using the common conjugated secondary antibody. In this method, the primary unconjugated antibody (monoclonal mouse anti-alpha tubulin or actin antisera) is allowed to bind the antigen (microtubules or microfilaments). A second tracer-conjugated antibody raised in another animal host and specific for the animal and immunoglobulin class of the primary antibody, is applied to the section and allowed to bind with the primary antibody. The use of colloidal gold as a marker system for immunocytochemistry was introduced by Faulk and Taylor (1971). It has found wide usage in ultrastructural immunolocalisation. The colloidal gold is the most popular metal tracer in use for many reasons which may be summarized as follows: Firstly, their shape and intrinsic high electron density make gold particles easily recognizable under EM. Secondly, they can be produced in various sizes and therefore, using particles of different diameter, it is possible to perform multiple immunolabelling. Thirdly, they are easy to prepare and can be stored for long periods of time. The size of the colloidal gold particle is also important. They can range from 1 nm to 60 nm, depending on the method of production. If large gold particles are used, there is the possibility that it will prevent some antigen-antibody reaction. Larger particles also have greater mass and this can cause them to be pulled away from the antibody during vigorous washing. Five nm particles will give a more accurate representation of the amount of antigen present and can be used for quantitative assessment. They have one major disadvantage, however, as they are not readily visualised at low magnification. As a result, 15-20 nm particles are more convenient for general application.[17] It was not widely used in light microscope immunoctyochemistry until the advantages of silver development were reported. In this method, the gold particles are enhanced by onion-skin like layers of metallic silver.[17]

For light microscopic study, we used 0.04% Triton X-100 in PBS to facilitate contact between the hydrophobic surface of the specimens and immunoreagents and 50 mM ammonium chloride for breaking the cross-linked protein to allow the binding between immunoreagent and the epitope.

Monoclonal mouse anti-alpha actin (1 : 50) and monoclonal mouse anti alpha tubulin (1 : 1,1000) antisera were used. These concentrations of antisera were recommended by many immunological studies of mouse's oocytes.[16,18] Therefore, this may be inappropriate concentration for the human oocytes, especially with intact zona pellucida. As this is the first study to use immunogold technique in the human oocytes, there have been no previous studies about the appropriate concentration of immunogold conjugated goat antimouse IgG for the human oocytes. The concentration used in this research was suggested for the mouse's oocytes by Vander Elst et al (1988) and the silver enhancement technique was undertaken using the methodology published by Robinson et al (1990).[16,17]

In postembedding immunocytochemistry, immunoreactions are carried out after fixation and embedding, i.e. directly on ultra-thin sections, which are normally mounted on nickel or gold grids, and most workers use an indirect immunostaining procedure and a gold-labelled antibody. The main advantages of postembedding immunogold techniques are : (a) there is no penetration problems since the cells are open to the immunoreactants and these are surface reactions: (b) the antigens are marked more precisely when particulate markers, i.e. colloidal gold particles are used.[19]

The type of embedding media employed is critical in the detection of some antigens. The activity of many antigens can be drastically reduced when conventional epoxy resins are used. This has led to the reemergence of acrylic resins, especially the cross-linked resin "Lowicryl" and "LR" (white and gold), which can withstand electron interaction in the microscope.[17] This is probably why the result of our immunocytochemical postembedding technique was not successful as the epoxy resin was used in this study.

In conclusion, the first part of this work described the application of the new method to handle and prepare the human oocytes for EM. The major advantage of this method is the reduction on time required to locate, section, stain and examine of the human oocytes.

The effects of MAF (SUZI, DISCO), sucrose, and sonic sword on cytoskeleton system of the human oocytes were studied by EM and immunocytochemistry. Immunogold and immunogold-silver staining techniques were applied to study the cytoskeleton of human oocytes.

Although the sample numbers are too small to permit statistical analysis of the data, subjectively it appears that microassisted fertilization (SUZI, DISCO), sucrose, and sonic sword may be the risk factors of the human oocytes' cytoskeleton abnormality, especially those exposed to combined risk factors or the direct disturbance of the cytoskeleton system produced by DISCO.

It is possible that, in the near future, ultrastructural studies will become an integral part of new reproductive technology because there is no other techniques available at present that can provide the quality of detail on human gametes and early embryos. This will be beneficial not only in the research but also in clinical practice, as it will enable the detection and help to explain some kinds of abnormalities of human's gametes and early embryos.

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CASE REPORT

Prenatal Diagnosis of Congenital Hypophosphatasia : A Report of 2 Consecutive Pregnancies

Theera Tongsong MD,*
Saipin Pongsatha MD,*
Sumalee Siriangkul MD.**

* *Department of Obstetrics and Gynaecology*

** *Department of Pathology, Faculty of Medicine, Chiang Mai University, Thailand*

ABSTRACT

Hypophosphatasia, an inherited autosomal recessive, is characterized by the demineralization of bones associated with deficiency of alkaline phosphatase. The incidence is 1 in 100,000 births. Accurate prenatal diagnosis of this lethal skeletal dysplasia is now possible, and the option of pregnancy termination can be offered.

A 27 year-old primigravida, 25 weeks of gestation, presented with large-for-date uterine size. The obstetric ultrasound showed polyhydramnios, poorly ossified and globular thin cranium. The soft skull could be compressed transabdominally with ultrasound tranducer. All of bony structures were delicate, and diffusely demineralized. However, vertebral bodies, both femurs, and humerus had some degree of ossification, shortened, and bowed but had no apparent fracture. The sonographic findings were most likely related to hypophosphatasia.

Two years later, the same patient presented to the antenatal clinic with the second pregnancy at 7 weeks' gestation. The screening ultrasound at 15 weeks revealed normal with exception for rather short femurs. The follow up ultrasound at 19 weeks showed a single fetus with micromelia without fractures, thin and poorly ossified cranium, ribs, vertebrae, and all of long bones. Only three abdominal vertebral bodies were somewhat ossified. Based on the sonographic findings and previous history of first pregnancy, the diagnosis of recurrent congenital hypophosphatasia was made.

Both pregnancies were electively terminated. Postnatal radiographs, autopsies and serum alkaline phosphatase levels confirmed the prenatal diagnosis.

Key words : congenital hypophosphatasia, prenatal diagnosis, ultrasound

Hypophosphatasia is one of the rare lethal short - limbed dwarfisms occurring in approximately 1 per 100,000 births.[1] It is characterized by the demineralization of bones associated with deficiency of alkaline phosphatase. It is inherited in an autosomal recessive pattern.[2] The homozygote has a severe deficiency of tissue and serum alkaline phosphatase and excessive urinary excretion of phosphoethanolamine. Because this condition is uniformly fatal, prenatal diagnosis is important, and the option of pregnancy termination can be offered at any time during gestation. The objective of this report is to demonstrate that prenatal diagnosis by ultrasound is possible for this lethal condition.

Case Report

First pregnancy

A healthy 27-year-old primigravida was admitted to Maharaj Nakorn Chiang Mai Hospital, at 25 weeks' gestation by last menstrual period due to recurrent premature contraction. Her prior medical and familial histories were unremarkable. The patient attended our antenatal clinic since 13 weeks' gestation.

On abdominal examination, the fundal height was 28 centimetres above pubic symphysis, large-for-date uterine size. Fetal heart sound was positive. Ultrasound examination was performed to detect anomalies in cases of premature contraction and polyhydramnios. The ultrasound examination showed single fetus with breech presentation, polyhydramnios, poorly ossified and globular thin cranium through which the sulci and gyri of the brain could be easily outlined. The soft skull could be compressed transabdominally with ultrasound transducer. All of bony structures were delicate, diffusely demineralized. However, vertebral bodies, both femurs, and humerus had some degree of ossification. All of long bones were shortened, and bowed but with no apparent fracture.

Prenatal diagnosis of lethal short-limbed skeletal dysplasia was made and was most likely related with hypophosphatasia. The pregnancy was terminated vaginally. A stillborn-male infant weighing 800 grams with globular, thin, soft and compressible calvarium, shortened limbs. Postnatal radiograph showed poorly ossified globular calvarium. All of bony structures was delicate, poorly ossified. Ulna, fibula, most of metacarpal, metatarsal and phalangeal bones could not be seen. However, vertebral bodies, both femurs, and humerus had some degree of ossification. All of long bones were shortened, and bowed but no apparent fracture.

Serum alkaline phosphatase level of the abortus was extremely low, but tissue alkaline phosphatase was not determined. Autopsy findings were also compatible with the diagnosis of severe hypophosphatasia.

Second pregnancy

Two years later, at age of 29 years, she presented to the antenatal clinic, Maharaj Nakorn Chiang Mai Hospital with the second pregnancy at 7 weeks' gestation. She was healthy. Routine prenatal serologic and haematologic tests were unremarkable. Physical examination at 7, 11 and 15 weeks of gestation revealed pregnancy size consistent with dates. Ultrasound screening due to previous child with congenital hypophosphatasia was performed at 15 weeks. The sonographic findings revealed normal with exception for rather short femurs (single fetus with BPD 30 mm, HC 10.7 mm, AC 10.2 mm, placenta grade 1, normal amniotic fluid volume).

The follow up ultrasound at 19 weeks showed a single fetus with cephalic presentation. The amount of amniotic fluid was within normal

limit. The fetus had a thin, poorly ossified cranium through which the sulci and gyri of the brain could easily be seen. The thorax was rather narrow. The ossification of ribs, vertebrae, and all of long bones were markedly decreased. Only three abdominal vertebrae appeared somewhat ossified. There was severe shortening of all limbs without evidence of any fracture or callus formation. Based on these sonographic findings and previous history of first pregnancy, the diagnosis of recurrent congenital hypophosphatasia was made. (BPD 40 mm, HC 12.7 mm, AC 13.2 mm, femur length 14 and 12 mm. The placenta was normal, grade 1 and the amniotic fluid volume was normal)

After counseling and psychological support had been given, the pregnancy was terminated with transcervical misoprostol. A stillborn female fetus weighing 310 grams was spontaneously expulsed. The postnatal appearance showed thin and soft calvarium with severe shortenting of all limbs.

Postnatal radiographs showed a poorly ossified calvarium, narrow thorax, severe shortening of all long bones, absence of ossiication of ribs, long bones and all of vertebrae except for three abdominal vertebral bodies. Serum alkaline phosphatase of the abortus was extremely low, but tissue alkaline phosphatase was not determined. The autopsy findings were compatible with congenital hypophosphatasia.

Discussion

Congenital (lethal) hypophosphatasia is a rare error of inborn metabolism characterized by abnormal bone mineralization with an extreme deficiency of alkaline phosphatase.[2,3] The mechanism for the development of bone fragility in this disease is not clearly understood. Alkaline phosphatase normally acts on pyrophosphatase and other phosphate esters which leads to the accumulation of inorganic phosphates, which are critical for the malformation of bone crystals. A deficiency in alkaline phosphatase leads to deficient generation of bone crystals. The major sonographic features of congenital hypophosphatasia are short-limbed dwarfism with thin, delicate bones and decreased bone echogenicity due to diffused hypomineralization.[4,5]

A precise prenatal differentiation of severe hypophosphatasia from other skeletal dysplasia may be difficult. The major causes of short-limbed dwarfism with hypomineralization of bones include hypophosphatasia, osteogenesis imperfecta type II, and achondrogenesis.[6] The common sonographic features of achondrogenesis are absent vertebral body and sacral ossification, while calvarium is rather completely ossified, in contrast to the echolucent skull in hypophosphatasia. The degree of moderate to severe micromelia and diffused hypomineralization in hypophosphatasia may resemble that of osteogenesis imperfecta type II. However, in contrast to the thickened bones of osteogenesis imperfecta type II, the extremity bones in hypophosphatasia tend to be delicate or may even be absent,[4-8] as were indeed the cases here. The sonographic findings of diffused demineralization, short-limb dwarfism, and thin delicate bones in our cases suggested severe hypophosphatasia rather than achondrogenesis or osteogenesis imperfecta. However, the definitive diagnosis could only be confirmed by postnatal radiograph and low serum alkaline phosphatatase.[3]

Sonographic diagnosis of congenital hypophosphatasia in first trimester may be impossible due to the fact that even in normal fetus incomplete ossification at this stage of development may be difficult to discriminate from the abnormal one. At what gestational age can the sonongraphic

diagnosis of congenital hypophosphatasia be confidently made is difficult to conclude. This report suggests that the evidence of skeletal dysplasia has been already appeared as early as 15 weeks' gestation. We believe that routine ultrasound screening at midpregnancy can diagnose this lethal condition. The patient with previous history of congenital hypophosphatasia should

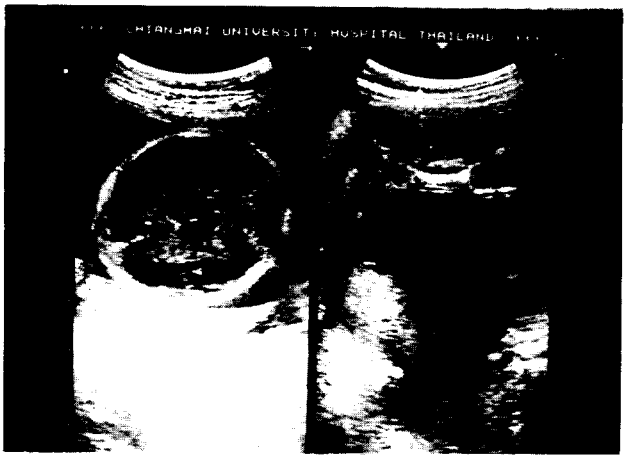

Fig. 1. (First pregnancy)

Left : The sonolucent skull through which sulci and gyri of brain could be seen.

Right : The longitudinal scan of lower extremity shows poorly ossified long bones and foot.

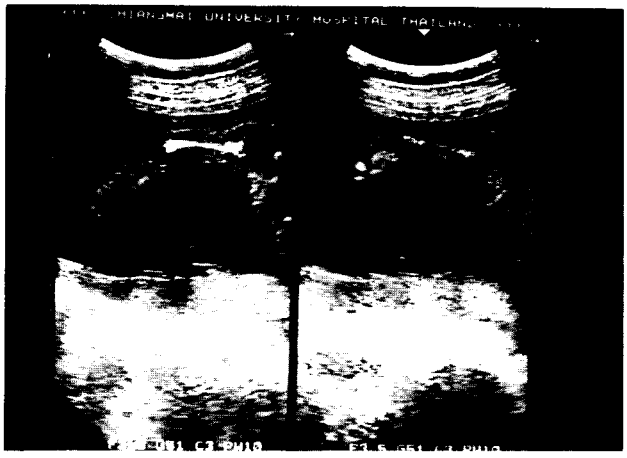

Fig. 2. (First pregnancy)

The longitudinal scan of upper extremity shows sonolucent long bones and irregular shape of ulna and radius.

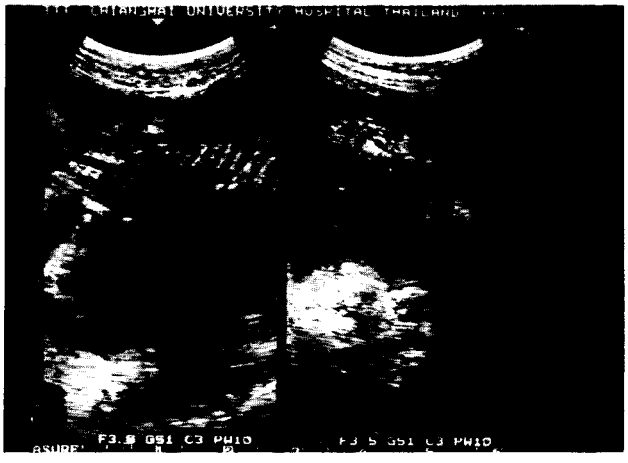

Fig. 3. (First pregnancy)

Left : The poorly ossified ribs.

Right : The poorly ossified bony structures of foot.

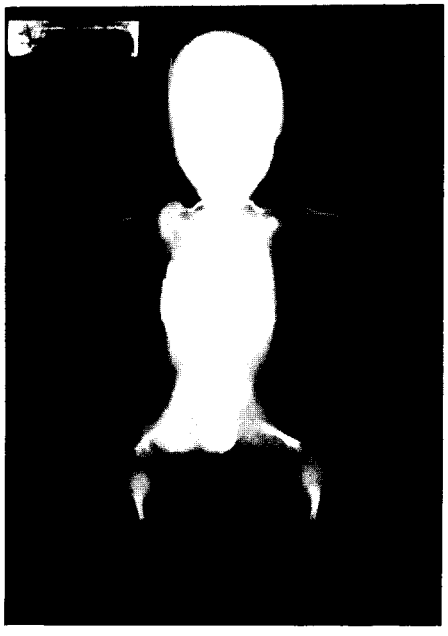

Fig. 4. (First pregnancy)

Postnatal radiograph confirms the prenatal findings.

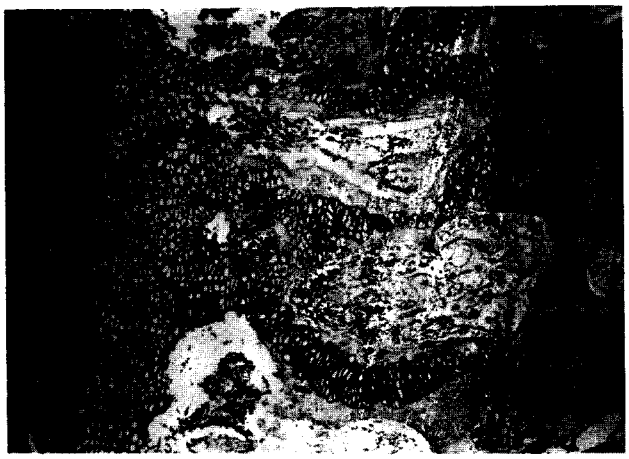

Fig. 5. (First pregnancy)
Microscopically, the zones of proliferative maturing and hypertrophic cartilage of the growth plate are widened and irregular. The cartilage also persists into the metaphysis with excessive unclassified osteoid.

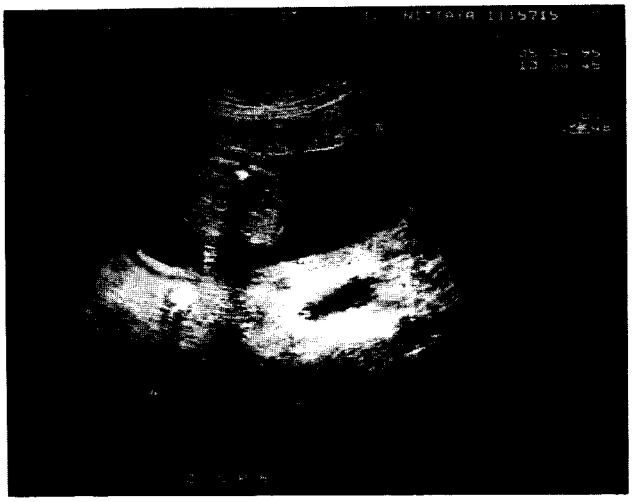

Fig. 6. (Second pregnancy)
Transverse sonographic scan of fetal abdominal spines shows the invisible posterior ossification centre and more echodense anterior body.

Fig. 7. (Second pregnancy)
The longitudinal scan shows poorly ossified vertebrae with exception for only three anterior bodies.

Fig. 8. (Second pregnancy)
The longitudinal scan of lower extremity shows poorly ossified and severe shortening long bones.

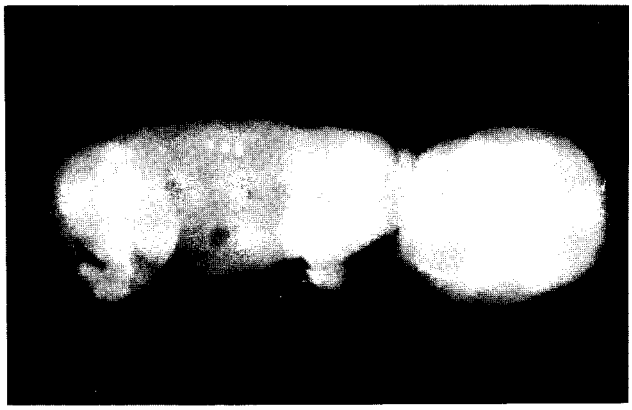

Fig. 9. (Second pregnancy)
Postnatal radiograph shows almost complete lack of mineralization of bony skeleton.

be sonographically screened at intervals from early second trimester.

Recently, prenatal diagnosis has been accomplished successfully by alkaline phosphatase assay on chorionic villus sample taken in the first trimester.[9] Congenital hypophosphatasia is an inherited autosomal recessive pattern[2] with a 25% chance of recurrence in the next pregnancy. Inspite of negative familial history, attempt must be made to exclude this condition in subsequent pregnancy. When an established confident diagnosis of this lethal skeletal dysplasia is made, the option of pregnancy termination should be offered to the patient.

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CASE REPORT

Pregnancy after Intracytoplasmic Injection of Sperm Obtained from Testicular Sperm Extraction : The First Case Report in Thailand

Jongjate Aojanepong MD,
Charoen Taweepolcharoen MD,
Karun Pongpipat MD,
Preecha Rungsaksangmanee MD,
Usanee Jetsawangsri MSc (ART),
Pongtorn Wattanasirisuk MD,
Seree Teerapong MD,
Piyada Wiratpong BSc.

Jetanin Institute for Assisted Reproduction, Bangkok, Thailand

ABSTRACT

Here we report a case in which fertilization and pregnancy was achieved after intracytoplasmic sperm injection (ICSI) of testicular sperm in an azoospermic patient with minimal spermatogenesis and maturation arrest. Eight oocytes fertilized normally out of 10 injected. Four pronuclear stage embryos were transferred into the fallopian tubes and the others were cryopreserved. Despite using nearly immotile sperm, pregnancy was still possible. So in association of ICSI and testicular sperm extraction (TESE) most cases of azoospermic patient can now be successfully treated.

Key words : intracytoplasmic sperm injection, testicular sperm, azoospermia

Intracytoplasmic sperm injection (ICSI) has been recommended as the method of choice in assisted fertilization for severe oligospermic patients.[1] The presence of only a few weakly motile sperm in a centrifuged ejaculate is sufficient to produce results equivalent to that of IVF in couples with normal semen.[1,2] However, in patients with secretory azoospermia, a few sperm can occasionally be recovered by testicular sperm extraction (TESE) for subsequent ICSI.

ICSI with epididymal and testicular extracted sperm gives fertilization and pregnancy rates similar to results using ejaculated spermatozoa.[3-5] Here we report a case in which fertilization and pregnancy was obtained from ICSI of testicular sperm in an azoospermic patient with severely impaired spermatogenesis and maturation arrest. This is, to our knowledge, the first pregnancy reported by the combined TESE/ICSI in Thailand.

Case Report

A couple with 5 years primary infertility was studied. The husband was 30 years of age and was found to be azoospermia from mump orchitis. His testicular biopsy result showed minimal spermatogenesis with maturation arrest and Leydig's cells were of usual appearance. Serum FSH and testosterone were normal. His wife was also 30 years of age and had normal menstrual cycles and normal tubal patency (both). After counseling the couple entered our IVF programme to try ICSI with testicular sperm.

A testicular biopsy was performed after local anaesthesia. A small piece of extruding testicular tissue was excised and put in 1-2 ml of HEPES-buffered Medicult medium in a small test tube. Content of the test tube was poured into a petri dish and the tissues were dissected thoroughly with 27 gauge needles under a dissecting microscope. After dissecting, the testicular tissue was checked under an inverted microscope for the presence of sperm cells. The dissection medium was aspirated carefully into a 1.5 ml Eppendorf tube. Any of sperm preparation method would have been unnecessary to be attempted since the number of spermatozoa was so few and motility was so weak. We had no choice but to pick the individual spermatozoon out of the field of debris, red blood cells and Sertoli cells for ICSI. So without further treatment, the testicular tissue solution was kept in an incubator at 37°C, 5% CO_2 until the moment of the injection procedure.

The female patient was prepared for egg retrieval after GnRH agonist suppression and hMG ovarian stimulation. Transvaginal egg retrieval was performed thirty-six hours after hCG administration. The cumulus and corona cells were removed by incubation for 30 sec in HEPES-buffered Medicult medium containing 80 IU/ ml of Hyaluronidase (type VIII) and by mechanical aspiration. Afterwards, the oocytes were observed under the inverted microscope and nuclear maturation was recorded. Until the moment of the injection procedure, oocytes were kept in 25 µl droplets of Medicult IVF medium covered by light-weight paraffin oil in an incubator at 37°C, 5% CO_2. ICSI was carried out on all oocytes that had extruded the first polar body (at metaphase II).

Just before the injection procedure, testicular suspension was centrifuge at 1,800 g for 5 min in Eppendorf tube. The supernatant was removed with a Pasteur pipette, after adding 0.2 ml of HEPES-buffered Medicult medium the pellet was gently resuspended.

Intracytoplasmic sperm injection was performed according to Palermo et al.[6] A single almost immotile spermatozoon was aspirated into the injection pipette and transferred to a clean droplet of 10% PVP solution. Debris and contaminated red blood cells were removed by repeated pipetting of the sperm in several clean area of the PVP droplet. The sperm cell was aspirated, tail first, into the injection pipette. The oocyte was secured by the holding pipette with the polar body positioned at 6 o'clock, then a single spermatozoon was injected into the ooplasm. The injection procedure was repeated

until all metaphase II oocytes were injected. The injected oocytes were washed in several drops of IVF Medicult medium and incubated in 25 µl of IVF Medicult medium under light-weight paraffin oil overnight at 37°C, 5% CO_2.

About 16 hours after the microinjection, the oocytes were observed for intactness and for the presence of pronuclei and second polar bodies. Fertilization was considered normal when two clearly distinct pronuclei were present. The pronuclear stage embryos were transferred into the fallopian tubes by laparoscopic procedure.

Results

Of the 10 metaphase II oocytes injected, 8 fertilized normally. Four pronuclear stage embryos were transferred into the fallopian tubes (2 embryos for each tube) and the other three were cryopreserved. A positive serum βhCG was detected on day 12. Five weeks after transferred, an ultrasound scan confirmed a twin pregnancy with positive fetal heart beat.

Discussion

The present report demonstrated that fertilization and pregnancy can be achieved by intracytoplasmic sperm injection of testicular sperm from an azoospermic man resulting from mump orchitis. Eventhough testicular sperm retrieved in this case were either immotile or, at the most, displayed shaking movements, a high fertilization rate was still possible. Epididymal transit may not necessary for testicular spermatozoa to acquire fertilizing ability since with intracytoplasmic injection we bypassed the zona pellucida and oolemma of the oocyte, thus allowing a testicular sperm with deficient motility or acrosome to fertilize an oocyte. Since the number and motility of testicular spermatozoa are so low, thus cryopreservation has not yet been feasible. Therefore, the husband has to undergo multiple biopsy procedures if subsequent cycles are needed. However, many testicular biopsies can be taken without major inconvenience.[3] In summary, the present case report shows that intracytoplasmic sperm injection of testicular sperm extraction can be attempted for azoospermic male with severely decreased spermatogenesis and can result in fertilization and pregnancy.

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Dr. Kobchitt Limpaphayom

Department of Obstetrics and Gynaecology

Faculty of Medicine, Chulalongkorn University

Chulalongkorn Hospital, Bangkok 10330, Thailand

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CASE REPORT

Remission of Recurrent Endometrial Cancer with Hormonal Therapy : Case Report and Review of Literature

Surintip Piamsomboon MD,*
Wichai Termrungruanglert MD,**
Andrzej P Kudelka MD,***
Vitaya Titapant MD,*
Creighton L Edwards MD,***
John W Whitt MD,****
John J Kavanagh MD.***

* *Siriraj Hospital, Mahidol University, Bangkok, Thailand,*

** *Chulalongkorn University Hospital, Bangkok, Thailand,*

*** *The University of Texas M .D. Anderson Cancer Centre, Houston, Texas,*

**** *Gulf Coast Cancer Centre, Wharton, Texas*

ABSTRACT

A case of stage II endometrial cancer which had recurred after radiotherapy and hysterectomy was manipulated with hormonal therapy. Oral megestrol acetate 160 mg per day induced partial response of measurable lesion. The duration of response was appreciated, 41 months. Later tamoxifen 40 mg per day orally also stabilized the progressive disease for another 18 months. Hormonal therapy has modest activity, low toxicity and should be considered in selected case of recurrent endometrial cancer

Key words : recurrent endometrial cancer, hormonal therapy

Recurrent and metastatic endometrial carcinoma has very poor prognosis. Currently, systemic therapy with either hormonal or cytotoxic agents has a palliative role only. Progestogen has been a major hormone used in advanced or recurrent endometrial carcinoma. Different progestogens, such as medroxy progesterone acetate (MPA), megestrol acetate (Megace) and hydroxy progesterone caproate (Delalutin), produce equivalent anti-tumour activity.[1,2] The

overall response rate, the duration of response and duration of survival on progestogen therapy are modest. However, occasional sustained remission and prolonged survival has been reported.[3]

Tamoxifen, the non-steroidal antiestrogen, can induce progestogen receptors in endometrial carcinoma.[4,5] Furthermore, it has anti-tumour activity in endometrial cancer.[6] It may be used as second line hormonal therapy when progesterone therapy fails.

We report a case of recurrent endometrial carcinoma who had prolonged remission of her disease with megestrol acetate. When her disease progressed, she was treated with tamoxifen which stabilized her progressive disease.

Case Report

A 67 year old white woman, para 5, abortion 1, developed recurrent endometrial cancer. Her tumour history began in March of 1990 when she presented to her gynaecologist with vaginal bleeding for one week. Pelvic examination revealed enlarged uterus, size equal to eight weeks' pregnancy. The cervix was 4 cm in diameter. There was a fungating tumour mass 1.5 cm in size filling the cervical os. The endometrial cavity sounded to 9.3 cm. Endometrial and cervical biopsies revealed endometrioid adenocarcinoma of the endometrium, grade II. The metastatic investigation showed no evidence of cancer outside the uterus. She was diagnosed as stage II, grade II, endometrial adenocarcinoma. The treatment consisted of whole pelvis radiation, 45 Gy divided into 27 fractions over 5 weeks, followed by single intracavitary Radium insertion. In July of 1990 (seven weeks after the completion of radiotherapy) she underwent exploratory laparotomy with extrafascial hysterectomy and bilateral salpingo-oophorectomy. Exploration of intraperitoneal cavity showed no evidence of tumour and no lymphadenopathy. The pathological examination revealed residual adenocarcinoma along the endometrial lining. The depth of myometrial invasion was less than 2 mm The other resected tissues were unremarkable.

In February, 1991 she began to have right flank pain. An intravenous pyelogram showed bilateral hydronephrosis and hydroureter. A computed tomography (CT) of abdomen and pelvis showed small amount of ascites. There was a soft tissue mass along the left side of the anterior abdominal wall. A CT guided fine needle aspiration of this mass revealed metastatic adenocarcinoma. Bilateral ureteral stents were placed to relieve the ureteral obstruction. She was then referred to the University of Texas M.D. Anderson Cancer Centre. On our initial evaluation, she reported mild intermittent right sided flank pain. Physical examination revealed fullness on both sides of the mid abdomen and minimal discomfort on deep palpation, which might represent residual bilateral hydronephrosis. There was no lymphadenopathy. Pelvic and rectal examinations were unremarkable. All pathology was reviewed and reported as well differentiated adenocarcinoma of endometrium with mucin production. The serum CA 125 and CEA were 1335.7 U/ml and 2292 ng/ml respectively. The serum creatinine was normal. The therapy was initiated with oral megestrol acetate 160 mg in four divided doses.

Two months later the serum CA125 and CEA dropped to 370 U/ml and 751 ng/ml respectively. The CT of abdomen and pelvis showed more than 50% decrease in the size of the metastatic tumour in the abdomen. She had no symptoms. She was in remission for 41 months with a good quality of life. Periodic physical examination and CT of abdomen and pelvis were

used to follow the persistent partial remission. She also had a good serologic response. The CA 125 level ultimately dropped to normal and the serum CEA remained at 120 - 180 ng/ml.

In September of 1994, a follow up CT scan of abdomen and pelvis demonstrated progression of the tumour. However she remained asymptomatic. Her CA125 level was normal, but the serum CEA became elevated to 300 ng/ml. Because of the good initial hormonal response of her disease, her treatment was changed to tamoxifen 20 mg orally twice a day. Her disease has again stabilized with no further progression for another 18 months.

Discussion

Generally, the therapeutic approach in recurrent metastatic endometrial carcinoma is based on the degree of tumour differentiation, inferred status of steroid receptors and the patients' performance status. The patient who has minimal disease with no symptoms, well differentiated tumours and positive steroid receptors is a good candidate for hormonal therapy. The advantages of this therapy are patients' tolerance, ease of administration, low toxicity and low cost.

Various types of progestogens have been tried in advanced or recurrent endometrial carcinoma with the overall response rate of 34%, as reviewed by Kauppila.[1] The duration of response ranged from 16 to 28 months and the average survival time was 18 to 33 months. Subsequent studies revealed lesser overall response rates due to the high proportion of patients with high grade tumours and differences in response criteria.[2,7,8] Thigpen et al[7,8] reported 14, 18 and 26% objective response rates with oral MPA 150, 200 and 1,000 mg/d respectively. The median duration of survival was 10.4 months. Podratz et al[2] performed a retrospective review of 155 patients and found objective response rates of 11, 9 and 12% for megestrol acetate, hydroxy progesterone caproate and medrogestone respectively. Interestingly, almost 40% of the patients had stabilization of their disease for three months or longer. Forty percent of patients with low grade tumours had an objective response. Overall, the 5 year survival rate was 8%.

Tamoxifen has been used in endometrial carcinoma with a widely variable response rate. The pooled data from Moore et al[6] revealed 22% response rate (range 0-53%) when 20 to 40 mg/d orally was used. The patients with low grade tumours, presence of steroid receptors, and prior response to progestin therapy had a higher response rate to tamoxifen than patients without these characteristics.[9] A downregulation of the progesterone receptor from prolonged progestogen therapy was considered as the cause of acquired progestogen resistance. Tamoxifen can increase the level of progesterone receptors but the clinical data failed to demonstrate the benefit of adding tamoxifen to progestogen in treating advanced or recurrent endometrial carcinoma.[9,10]

Gonadotropin releasing hormone (GnRH) analogs represent another hormonal modality which has been studied in various gynaecologic malignancies. Of 17 patients with progestogen resistant advanced or recurrent endometrial cancer, 6 patients (35%) had an objective response including 1 patient with a complete response. The median duration of response was 24 months.[11]

The advanced age, poor performance status and coexisting illness in patients with endometrial cancer frequently limit the use of chemotherapy. Single agent trials which demon-

strated objective responses of more than 20% were Cisplatin,[12] Doxorubicin,[13] and Carboplatin.[14,15] The median time to progression and median duration of survival were short lasting only 2 to 4 months and 4 to 8 months respectively. The combination of Cisplatin, Doxorubicin and Cyclophosphamide with or without progestogen was most active with an objective response rate of up to 53% but the duration of response was only 4-6 months and the survival duration was less than 12 months.[16,17] Furthermore, moderate to severe toxicity occurred in almost half of the patients treated with this combination chemotherapy.

Current systemic therapy for recurrent metastatic endometrial carcinoma is palliative. Though hormonal therapy has modest activity, selection of patients may respond and maintain good quality of life. Accordingly, hormonal therapy should be considered in patients with advanced or recurrent endometrial carcinoma.

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Department of Obstetrics and Gynaecology

Faculty of Medicine, Chulalongkorn University

Chulalongkorn Hospital, Bangkok 10330, Thailand

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CASE REPORT

Endocervical - Like Mucinous Borderline Tumour of the Ovary : Case Report with the Clinical and Histopathological Study

Toshimitsu Tohya MD,
Yoshihiro Honda MD,
Katsuyasu Ishikawa MD.

Department of Obstetrics and Gynaecology, Minamata City General Hospital, Minamata, Kumamoto 867, Japan

ABSTRACT

Clinical and histopathological features of an ovarian endocervical - like mucinous borderline tumour (EMBT) are studied. A 31 - year - old women had a right cystic ovarian tumour with a portion of papillary proliferation at the inner wall. Peripheral blood carbohydrate antigen 125 (CA125) level was 44 U/ml, carcino-embryonic antigen (CEA) was 1.4 ng/ml, which were within a normal range. Carbohydrate antigen 19-9 (CA19-9) was 390 U/ml, an abnormally high level. A right adnexectomy was performed. Histopathological examinations showed that the tumour was an ovarian endocervical - like mucinous borderline tumour. The histopathological features of EMBT are papillary proliferation and the epithelium resembling endocervical cells. Additionally, immunohistochemical staining showed that the specimens were positive for CA19-9 and negative for CA125 and CEA. She is alive and well 2 years and 10 months after the operation.

Key words : ovarian mucinous tumour, endocervical type, borderline malignancy, CA 19-9, immunohistochemistry

Recently, ovarian mucinous tumours of borderline malignancy are subclassified in three categories : (1) endocervical type, (2) intestinal type, (3) mixed type. One of them, ovarian endocervical - like mucinous borderline tumours (EMBTs) have been initially termed mullerian mucinous papillary cystadenomas of borderline malignancy by Rutgers and Scully.[1] EMBTs account for approximately 15% of ovarian mucinous borderline tumours (MBTs). There are still a few literatures on EMBTs.[1-3] We report clinical and histopathological features of a case of EMBT.

Case Report

A 31 - year - old housewife, para 2, complaining of lower abdominal pain since the beginning of January, 1991. She did not have remarkable family and past history. After gynaecological and ultrasound examinations she was suspected to have an ovarian tumour, and referred to our hospital on February 14, 1991. Gynaecological and ultrasound examinations revealed a right cystic ovarian mass of 7.5 x 5.3 x 5.3 cm, and an excrescence was seen in a part of the inner wall. No abnormal findings were seen by haemogram, biochemical examinations of the blood, chest X-ray, ECG, and pyeloureterography. The tumour markers, CA 19-9 was 390 U/ml, which was abnormally high. CA 125 was 30 U/ml, and CEA was 1.4 ng/ml, both values were within normal ranges. She was diagnosed to have an ovarian tumour, and underwent a laparotomy. Right ovarian tumour was found, the uterus and left ovary were normal looking. Right adnexectomy was performed. The tumour was unilocular, inner wall was smooth except a portion of solid excrescence was observed. She is alive and still free of disease 2 years and 10 months after the operation.

Histopathological Findings

Haematoxylin-eosin (HE) stain of the sections of this tumour mostly revealed mucinous cystadenoma, and a portion of papillary structures was presented (Fig. 1, 2). The epithelium of the papilla mimicked that of the cervical glands (Fig. 3). The nuclei were on the basal side, stratification and atypicality of the nuclei were partly seen (Fig. 3, 4). Inflammatory cellular infiltration was seen in the stroma (Fig. 4). Periodic-acid-Schiff (PAS), alcian blue, and mucicarmine, Grimelius stainings and immunohistochemical stainings of CA 19-9, CA 125 and CEA were then performed. The specimens were positive for PAS, alcian blue and mucicarmine stainings, and negative for Grimelius. Additionally, immunohistochemical staining showed that the specimens were positive for CA19-9 and negative for CA125 and CEA.

Discussion

Ovarian EMBT of a 31-year-old female patient is presented. Unique feature of our case report is the tumour marker findings. Haematologically, CA125 and CEA values were normal and CA19-9 value was abnormally high. Additionally, immunohistochemical staining of this tumour sections showed positive for CA19-9 and negative for CA125 and CEA. Rutger and Bell found that EMBTs and mixed-epithelial MBTs were positive for CA19-9, on the contrary intestinal-type MBTs were positive for CEA.[3] We had reported that intestinal-type mucinous tumour tend to be positive for CEA immunohistochemically.[4] These tumour markers findings may be useful to distinguish the three types of MBTs.

Histogenesis of ovarian mucinous tumours was obscure and complex. Concerning the MBTs, endocervical type and mixed-epithelial type are regarded as of mullerian origin. But the histogenesis of intestinal type has not yet been established. It is interesting that MBTs of endocervical-type and mixed-epithelial type show positive for CA19-9 and intestinal type show positive for CEA. Histogenesis of mucinous tumours may be more understandable by studies of these tumour markers.

Ovarian mucinous tumours account for 15 to 25% of all ovarian tumours. Of all mucinous tumours, about 85% are benign, 6% borderline, and 9% invasive.[5] Recently MBTs are subclassifed in three categories (endocervical, intestinal, or mixed type).[6] EMBTs account for approxi-

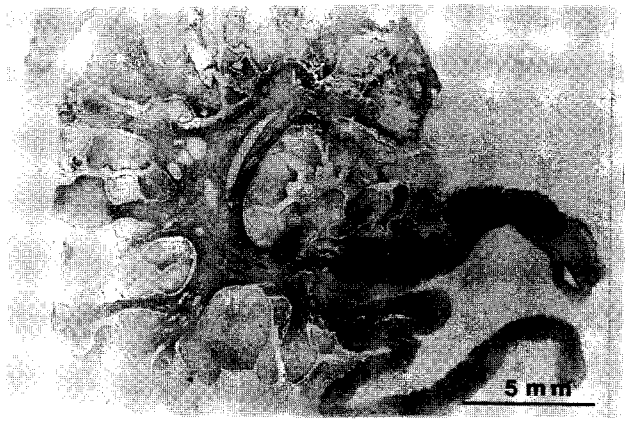

Fig. 1. Papillary projection in the inner wall of the tumour (5x).

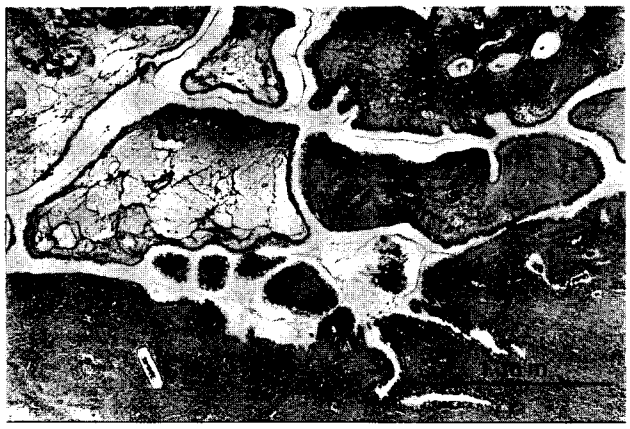

Fig. 2. Microscopic findings of the papilla (40x).

Fig. 3. The endocervical type epithelium is noted (200x).

Fig. 4. Indifferent cells with eosinophilic cytoplasm showing stratification and tufting. Inflammatory - cells in the stroma are also noted (400x).

mately 15% of all MBTs.[2] Still, there are few literatures on EMBTs.[1-3] Clinically, ovarian EMBTs often affected young women (average age of 34), often occurred in the ovaries bilaterally, and consisted of unilocular cystic tumour with a solid tumour in a part of the inner wall.[1] Our case had these features, including ultrasound and macroscopic findings.

Ovarian serous borderline tumours have characteristic papillary proliferation, but it is rare to manifest as papillary proliferation as in MBTs. Histological features of EMBTs were papillary structure, the epithelium resembled that of the cervical glands, and no evidence of intestinal differentiation (goblet, argentaffin, or Paneth cells) was seen. Inflammatory cellular infiltration was seen in the stroma in every cases. EMBTs are characterized by the presence of endocervical type epithelium. Its histological diagnosis should be done precisely.

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REVIEW

Aspirin and Prevention of Pre-eclampsia : The Current Situation

Yongyoth Herabutya MRCOG.

Department of Obstetrics and Gynaecology, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand

Pre-eclampsia remains an important cause of maternal and perinatal mortality and morbidity. Because the final cause of pre-eclampsia is obscure, early delivery and the control of blood pressure with various antihypertensive agents are the only modalities to treat pre-eclampsia. The development of pre-eclampsia begins with a loss of vascular refractoriness to vasoactive agents, followed by vasoconstriction. A functional imbalance between vasodilator and vasoconstrictor eicosanoid products appears to be of major importance in causing this loss of vascular refractoriness. Patients who develop pre-eclampsia exhibit a smaller increase in prostacyclin (PGI_2) biosynthesis than normal and a reduction in the urinary excretion of PGI_2 metabolites precedes the development of clinical disease. Thromboxane A_2 (TXA_2) biosynthesis is increased in pre-eclampsia, and the urinary excretion of TXB_2 metabolites correlates with severity of the pre-eclampsia disease process.[1] Aspirin inhibits platelet thromboxane production[2] and, in low doses, does not alter endothelial cell prostacyclin production, thereby shifting the balance of these factors towards vasodilatation and inhibition of platelet aggregation.

In essence, because low-dose aspirin inhibits thromboxane production whilst leaving prostacyclin production intact, both vasoconstriction and platelet aggregation could be prevented in pre-eclampsia. This is a simplistic view because of the large number of factors regulating vascular tone and platelet function but it provides a reasonable rationale to test aspirin in the prevention of pre-eclampsia.

Randomized trials

It is not the purpose of this article to review every study of aspirin in pregnancy, but rather to consider these controlled trials which have been published in referred journals. The focus is on the ability of aspirin to reduce perinatal mortality and intrauterine growth retardation (IUGR) and to prevent proteinuric pre-eclampsia. These randomized trials under discussion are summarized in table 1.[3-16] The points are as follow :

As a group, the small trials conducted before 1993 showed a substantial benefit of aspirin in preventing pre-eclampsia.[3-8] Later, larger trials failed to show the same benefit. In the Italian study,[9] and in the American study,[11] both showed no differences in the frequency of

Table1. Studies of Aspirin in Pregnancy

Year	Author	Entry criteria	Aspirin dose (mg)	Gestation at enrollment (wks)	Number of subjects	Prevalence of Pre-eclampsia (%)	Maternal outcome (aspirin group)	Fetal outcome (aspirin group)
1985	Beaufils[3]	Prior stillbirth/severe IUGR Essential hypertension	150*	12	102	34.8	No cases of PE	Reduced fetal loss/ IUGR
1986	Wallenburg[4]	Angiotensin II sensitivity	60	28	46	30.4	90% reduction in PE	No benefit
1989	Schiff[5]	Roll over test	100	28-29	65	22.6	80% reduction in PE	Longer gestation
1989	Benigni[6]	Prior stillbirth/severe IUGR Prior early onset PE Essential hypertension	60	12	33	0	No benefit	Longer gestation Higher birthweight
1990	McParland[7]	Doppler ultrasound	75	24	100	19	83% reduction in PE	No benefit
1991	Uzan (EPREDA)[8]	Prior stillbirth/severe IUGR	150**	15-18	230	11	75% reduction in PE	Higher birthweight less IUGR
1993	Italian Study Group[9]	At risk for PE	50	16-32	1106	2.7	No benefit	No benefit
1993	Hauth[10]	Healthy nulliparous	60	24	605	5.6	68% reduction in PE	No benefit
1993	Sibai[11]	Healthy nulliparous	60	13-26	3135	6.3	28% reduction in PE	No benefit
1993	Viinikka[12]	Essential hypertension/ prior severe PE	50	12-18	208	11	No benefit	No benefit
1994	CLASP[13]	At risk for PE/IUGR	60	12-32	9364	7.6	12% reduction in PE	14% reduction in preterm delivery
1995	Kyle[14]	Angiotensin II sensitivity	60	28	495	11	No benefit	No benefit
1996	Herabutya[15]	Healthy nulliparous	60	18-24	1348	2.9	27% reduction in PE	No benefit
1996	ECPPA[16]	High risk for PE/IUGR Chronic hypertension	60	12-13	1009	6.1	No benefit	No benefit

* included dipyridamole ** aspirin alone and aspirin + dipyridamole. PE = pre-eclampsia ; IUGR = intrauterine growth retardation

pre-eclampsia or IUGR. However, the finding in the American study[11] that the rate of abruptio placentae in aspirin group (0.7%) was significantly ($p < 0.01$) higher than in the placebo group (0.1%) caused much alarm. The other two small trials came in 1993 contradicted each other.[10,12]

The so called CLASP trial[13] (Collaborative Low-Dose Aspirin Study in Pregnancy) was run in 213 centres in 1988-92. This was the largest collaborative study ever undertaken in obstetric management, 9364 pregnant women were randomized to 60 mg of aspirin or matching placebo. Participating obstetricians could start this treatment at will when they felt it appropriate and no strict inclusion criteria were followed. As a consequence, aspirin was initiated for true prophylaxis against pre-eclampsia in only 74%, whereas the other causes for treatment included

prophylaxis or treatment against IUGR, and treatment of established pre-eclampsia. Aspirin use was associated with nonsignificant reduction in proteinuric pre-eclampsia, and did not protect against IUGR. It was further noteworthy that the risk of abruptio placentae was not increased. The other studies that followed did not show any benefit or any increase in abruptio-placentae either.[14-16] One study,[14] in particular, used the angiotensin sensitivity test and low-dose aspirin concluded that the angiotensin sensitivity test is not an effective screening test for pre-eclampsia nor does low-dose aspirin prevent pre-eclampsia. The other study[16] modelled along the lines of CLASP, considering all high risk pregnant women as a single group concluded that routine prophylactic administration of low-dose aspirin did not prevent pre-eclampsia. The same conclusion was also reached with the only one study from Thailand.[15]

Many earlier reports pointed to the benefits of low-dose aspirin, so why has this promise not been realized ? First the more recent studies are much larger and have therefore been able to address the issue comprehensively.[9,11,13,15,16] Second, there is a natural tendency for papers with positive results to be published at the beginning especially in the subject that stirs a vast interest. Third, and perhaps most important, in many of those studies that suggested striking benefits with low-dose aspirin, high-risk patients were selected by use of predictive tests such as the angiotensin II infusion test,[4] rollover test,[5] and uteroplacental Doppler.[7] This result does not represent so much a failure of its pharmacological action in correcting the thromboxane and prostacyclin ratio, but may be due to the fact that platelet activation is just only one part of the complex pathogenesis of pre-eclampsia which is still poorly understood until this day.

What can be recommended on the basis of existing studies ?

1. The results of available trials, at present, do not support the wide spread routine prophylactic or therapeutic use of antiplatelet therapy in pregnancy among all women judged to be at risk of pre-eclampsia or IUGR.[17]

2. On the other hand, prophylactic low-dose aspirin would benefit high risk pregnancy, although there is no definite proof for recommending this, and only if they can be identified early in pregnancy (ideally at less than 20 weeks' gestation). In recommending this, one acknowledged the lack of proven benefit but a significant trend towards benefit. This approach is based on the data from CLASP. Unfortunately an accurate predictive test for pre-eclampsia and IUGR suitable for routine clinical use is not available.[18] Prediction and prevention are inextricably linked, the solution to both these problems lies in the complex pathophysiology of pre-eclampsia that no one has unravel.

3. The low-dose aspirin prophylaxis should not be used in healthy nulliparous women,[11,15] women with mild chronic hypertension,[12,16] and women with established pre-eclampsia.[13]

4. There is still a question about the benefit of low-dose aspirin in the following groups of women : women with underlying renal disease with accompanying hypertension, proteinuria or impaired glomerular filter rate at conception ; women with multiple pregnancy ; women with severe essential hypertension at conception ; women with insulin dependent diabetes before conception, and women with a family history (mother and at least one sister) of severe pre-eclampsia. These groups should be submitted to further trial of aspirin. The findings of the

low-dose aspirin studies so far provide sufficient rationale to support further trials of prophylactic aspirin in these more highly selected subgroups of women considered at substantial risk of proteinuric pre-eclampsia or fetal loss.

5. We have learned from these trials that low-dose aspirin is generally safe. This was confirmed by follow up at 12 and 18 months of age of cohorts of surviving children whose mothers participated in a large randomized trial of 60 mg aspirin (CLASP) that low dose aspirin is generally safe, at least in respect of congenital malformation, major motor deficit and severe neuromotor or developmental delay identifiable in early childhood.[19]

The future trend

The work to elucidate the pathogenesis of these conditions has been continued. Undoubtedly the aetiology of pre-eclampsia is to be found very early in pregnancy. The morphological evidence is that pre-eclampsia is associated with failure of trophoblastic invasion of spiral arteries early on in the pregnancy. Whatever the mechanism of impaired trophoblastic invasion, the result is narrowed spiral arteries and subsequent placental ischaemia. One proposal is that such abnormal placentae release a factor or factors that bring about widespread endothelial cell activation leading to the multisystem dysfunction that characterizes pre-eclampsia.[20] Results from studies on the effect of sera from pre-eclamptic women on cultured human umbilical vein endothelial cells support the theory that pre-eclampsia is an endothelial cell disorder.[21]

What aspirin trial expected ?

A trial of prophylaxis in high risk women with chronic hypertension, previous pre-eclampsia, multiple gestation or diabetes mellitus is under way under the guidance of National Institute of Health (USA). Two further studies are under way in the West Indies and Australia. The one in Brazil has already been published.[16] The aspirin may yet find a place for prophylaxis treatment of women at high risk of developing early-onset pre-eclampsia including those with chronic hypertension, renal disease, multiple pregnancy, diabetes mellitus, previous severe pre-eclampsia and eclampsia.

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GUIDELINES

Committee for the Study of Ethical Aspects of Human Reproduction

International Federation of Gynecology and Obstetrics (FIGO)

GUIDELINES REGARDING INFORMED CONSENT

1. The obligation to obtain the informed consent of a woman before any medical intervention is undertaken derives from respect for her fundamental human rights. These rights have been widely agreed on and are laid down in such documents as the Universal Declaration of Human Rights (1948); the twin International Covenants on Civil and Political Rights and Economic, Social and Cultural Rights (1975) ; the International Convention on the Elimination of All Forms of Discrimination Against Women (1979) ; and the International Convention on the Rights of the Child (1989). Sexual and Reproductive Human Rights have also been identified by the International Conference on Population in Cairo (1994), and re-affirmed by the Fourth World Conference on Women in Beijing (1995).

2. The following definition of informed consent flows from these human rights and is endorsed by the FIGO Committee for the Study of Ethical Aspects of Human Reproduction :

"Informed consent is a consent obtained freely, without threats or improper inducements, after appropriate disclosure to the patient of adequate and understandable information in a form and language understood by the patient on :

a) the diagnostic assessment :

b) the purpose, method, likely duration and expected benefit of the proposed treatment :

c) alternative modes of treatment, including those less intrusive, and

d) possible pain or discomfort, risks and side-effects of the proposed treatment."

3. Although these criteria are clear, to implement them may be difficult and time consuming, for example where women have little education, or where very unequal power relationships in a society mitigate against women's self determination. Nevertheless these difficulties do not absolve physicians caring for women from pursuing fulfilment of these criteria for informed consent. Only the woman can decide if the benefits to her of a procedure are worth the risks and discomfort she may undergo. Even if, for example other family members feel they should make the decision, it is the ethical obligation of the physician to ensure her human right of self determination is met by the process of communication that precedes any informed consent.

4. It is important to keep in mind the fact that informed consent is not a signature but a process of communication and interaction.

5. The opinion of children or adolescents on a medical intervention should be assessed within the limitations posed by their level of development, age or understanding.

6. Even if a woman is unable to decide for herself because of mental incapacity or mental retardation, nevertheless she must be involved in the decision-making process to the fullest extent her capacity allows, and her best interests must be taken into account.

7. If physicians for reason of their own religious or other beliefs do not wish to fulfil the above criteria for informed consent because they do not want to give information on some alternatives; they have, as a matter of respect for their patient's human rights, an ethical obligation to make an appropriate referral so she may obtain the full information necessary to make a valid choice.

VIOLENCE AGAINST WOMEN

1. Violence against women is one reflection of the unequal power relationship of men and women in societies. Reflections of this inequality include marriage at a very young age, lack of information or choice about fertility control and forced pregnancy within marriage.

2. Violence against women is condemned, whether it occurs in a societal setting (such as female genital mutilation) or a domestic setting (such as spousal abuse). It is not a private or family matter. Violence against women is not acceptable whatever the setting and therefore physicians treating women are ethically obligated to :

i. Inform themselves about the manifestations of violence and recognise cases.

Documentation must take into account the need for confidentiality to avoid potential harmful consequences for the woman, and this may need separate, non-identifiable compilation of data.

ii. Treat the physical and psychological results of the violence.

iii. Affirm to their patients that violent acts towards them are not acceptable.

iv. Advocate for social infrastructures to provide women the choice of seeking secure refuge and ongoing counselling.

3. The physical, financial and social vulnerabilities of women are fundamentally harmful to the future of a society. Not redressing them fails to prevent harm to subsequent generations and contributes to the cycle of violence. Physicians treating women therefore have an obligation to:

i. Affirm women's right to be free of physical and psychological violence, particularly sexual violence, examples of which range from war crimes in conflicts between states to sexual intercourse without consent within marriage.

ii. Advocate for non-violent resolutions in relationships by enlisting the aid of social workers and other health care workers where appropriate.

iii. Make themselves, and others, aware of the harmful effects of the embedded discrimination against women in social systems.

4. There is a need for wider awareness of the magnitude of the problem of violence against women. Physicians are uniquely placed to assist in this. Only if a problem is recognised can it be addressed. There is therefore a duty for professional societies and physicians to publicize information about the frequency of types of violence against women.

ETHICAL ASPECTS IN THE MANAGEMENT OF THE SEVERELY MALFORMED FETUS

1. The Committee agreed that a woman carrying a severely malformed fetus had the ethical right to have her pregnancy terminated.

2. The qualification "severe" is used in this context to indicate malformations that are either potentially lethal or whose nature is such that even with medical treatment they are likely, in the view of the parents and their medical advisors, to result in unacceptable mental and/or physical disability.

3. The Committee felt that termination of pregnancy which resulted in the survival of a malformed infant with the added burden of prematurity was ethically unacceptable. Therefore most careful consideration should be given to the use of termination once a non-lethally malformed fetus may have become capable of independent life.

4. Because of legal, religious or other reasons, termination of pregnancy may not be an available option. In such circumstances, it is especially important to counsel and to seek informed consent on the use of diagnostic techniques such as ultrasound examination, that may reveal fetal malformation. At the same time, enquiry should be made as to the information the woman wishes to be conveyed to her should fetal malformation be fortuitously diagnosed or suspected.

5. It is unethical to permit the sex of the fetus to influence the decision to terminate a pregnancy when the malformation is non sex-related.

6. In multiple pregnancies involving both malformed and normal fetuses, the right of the normal fetus to survive should take precedence in decision making, except of course in the rare instance of the mother's health being put at risk.

7. In the event of the parents disagreeing on the course of action to be adopted, the woman's view should take precedence in decision making.

8. When the parent's medical advisor is unable to accept their request for the termination of pregnancy, he/she has a duty to advise them to seek a second opinion.

9. The decision to terminate a pregnancy should rest primarily with the parents. No medical or governmental coercion for financial or demographic reasons should be brought to bear on them.

10. Following termination of pregnancy the medical team has, with parental consent, a duty to confirm and document the fetal malformation (e.g. postmortem examination, chromosomal studies, etc.) and to inform and counsel the parents.

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Note : 1 puff = 150 µg.

Composition: Each bottle contains, in 10 g aqueous solution, 15.75 mg buserelin acetate, equivalent to 15 mg buserelin, as active ingredient, and benzalkonium chloride as preservative.

Indications: Endometriosis (unless the disease preimarily requires surgical treatment). The diagnosis must be confirmed.

Contraindications: Pregnancy. Lactation. Hypersensitivity to buserelin acetate or benzalkonium chloride.

Precautions: It is recommended to exclude pregmancy before starting treatment. Suprefact E is excreted in small quantities in the mother milk. Suprefact E should not be prescribed to lactating mothers although negative effects on the child have not been observed. Patients known to suffer from depression must be carefully monitored during treatment with Suprefact E

Adverse reactions: Threatment with Suprefact E is based upon the principle of suppressing the production of oestrogens throughout treatment. An episode of uterine bleeding resembling menstruation usually occurs in the first few weeks of treatment. In occasional cases, bleeding may also occur during the further course of treatment. As a result of oestrogen withdrawal, patients may suffer menopausal symptoms, such as hot flushes, increased sweating, dry vagina, decrease in libido, decrease in bone density (after several months of Suprefact E treatment, a loss of bone mass may occur). The following symptoms cannot be clearly attributed to hormone suppression: Headache (of migrainous type, in rare instances), fatigue, dizziness, palpitation, sleep disorders, nervousness, depressed moods, changes in weight, tenderness of the breasts with changes in size, changes in body hair, acne, dry skin, occasionally vaginal discharge. Nausea, vomiting, diarrhoea, constipation and stomach pain, pains in the back, limbs and lower abdomen have also been observed. Hypersensitivity reactions such as reddening of the skin and lesions of urticarial type may occur in isolated cases. Oedema of the face or extremities (arms and legs) has been observed occasionally. Because of its nasal use, irritation of the nasal mucosa is possible and may sometimes lead to nosebleeds. Manifestations of eye irritation may develop in wearers of contact lenses.

Interactions: No interactions with other drugs have been reported.

Dosage and administration: Unless otherwise prescribed, the daily dose of buserelin is 0.9 mg, regardless of body weight. Before starting Suprefact E treatment, patients should discontinue oral contraceptive pills. Barrier methods of contraception should be used during the first two months of treatment (e.g. diaphragm, condom). Treatment should be started on the first or second day of menstruation in order to exclude the existence of pregnancy as far as possible. It is unlikely that pregnancy will occur in the later stages of treatment, if the recommended doses are taken regularly. However, if treatment is interrupted, even for a few days, ovulation may occur and the patient may become pregnant. In the event of pregnancy, Suprefact E must be discontinued immediately, and the doctor should be consulted promptly. The usual duration of treatment is six months. It should not exceed nine months. Treatment should be repeated only after the attending doctor has carefully weighed the therapeutic benefit against possible risks, since additive effects cannot be excluded in patients with a loss of bone mass. Further information available on request

Hoechst Thai Ltd. Pharmaceutical Dept.
302 Silom Rd. Bangkok 10500 Tel. 233-2981

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GUIDELINES

Antenatal Corticosteroids to Prevent Respiratory Distress Syndrome

Scientific Advisory Committee of the Royal College of Obstetricians and Gynaecologists

1. INTRODUCTION

Respiratory distress syndrome (RDS) affects 40-50% of babies born before 32 weeks.[1] Evidence has been available since 1972[2] that the administration of corticosteroids prior to preterm delivery reduces the incidence of RDS. However, the use of antenatal corticosteroid therapy has been hesitant. An audit of preterm babies born in district general hospitals at a gestational age of less than 31 weeks in 1992 revealed that only 35% had antenatal exposure to corticosteroid therapy.[3]

2. EFFECTIVENESS

A meta-analysis of fifteen randomized controlled trials[4] indicates that antenatal corticosteroid therapy reduces the incidence of RDS. There is an associated reduction in the risk of neonatal death and intraventricular haemorrhage. The efficacy of neonatal sufactant therapy is enhanced by antenatal exposure to corticosteroids.[5] There is evidence of a benefit in all major sub-groups of preterm babies irrespective of race or gender.

GESTATIONAL AGE

While there is no evidence that the benefits are confined to preterm babies of a specific gestational age, an analysis of the "number needed to treat" suggests that after 34 weeks, 94 women will need to be treated to prevent one case of RDS, while before 31 weeks one case of RDS is prevented by every five women treated.[6]

TREATMENT-DELIVERY INTERVAL

The effect of treatment is optimal if the baby is delivered more than 24 hours and less than seven days after the start of treatment. However, there is a trend toward a benefit in babies delivered before and after the optimal treatment interval has elapsed.

MULTIPLE PREGNANCY

A statistically significant reduction in the incidence of RDS has not been demonstrated in preterm babies born following multi-fetal pregnancy. It is not known whether this is due to small numbers or to sub-therapeutic drug levels, perhaps secondary to plasma volume expansion.[7]

ECONOMIC EFFECTS

The cost and duration of neonatal intensive

care is reduced following corticosteroid therapy. However, the overall economic effect of antenatal corticosteroids will be influenced by the potential increase in survival in very low birth weight babies and by the use of surfactant. Simpson[8] calculated that an increase in use from 15% to 60% in babies of less than 2,000 grams would result in an annual saving of $157m. Mugford et al[9] predicted a more modest saving to the NHS, although the costs of treating low birth weight infants remain considerable.[10,11]

3. SAFETY

Survivors from three of the randomized trials have been followed up to between six and 12 years of age.[12-14] No adverse neurological or cognitive effects have been observed. Corticosteroid use does not appear to increase the risk of either fetal or maternal infection regardless of whether the membranes are ruptured or not at the time of treatment. One trial reported an excess of fetal deaths in women with severe proteinuric hypertension. All cases in which stillbirth occurred had been complicated by proteinuria of more than two grams per 24 hours for more than two weeks before intrauterine death occurred. Women with either insulin-dependent diabetes or gestational diabetes were not entered into randomized controlled trials of antenatal corticosteroid therapy so there is no evidence that antenatal corticosteroid therapy is either safe or effective in these circumstances. In view of the adverse effects of maternal hyperglycaemia on fetal lung maturation it is possible that any benefit of corticosteroids could be offset by corticosteroid-induced hyperglycaemia.[15]

4. REPEATED DOSES

It is important to emphasise that all evidence concerning safety and immediate and long-term side effects is derived from the randomized trials where a single course of treatment was administered. There are no randomized trials of repeated doses of antenatal corticosteroid therapy. The practice of repeating the course of treatment weekly has arisen in cases where the risk of preterm delivery persists or recurs following the initial treatment. The theoretical risks of this approach include some long-term effects on cognitive or neurological development that did not occur in the randomized trials which dealt with single courses of treatment only. Impaired glucose tolerance, osteoporosis and depression of either fetal or maternal adrenal function are other potential risks. Decisions to repeat treatment should be made individually, based on an assessment of the likelihood of delivery, and the risk of respiratory distress syndrome at a given gestational age. Where possible, amniotic fluid phosphatidyl glycerol should be estimated in order to restrict the need for repeated doses of corticosteroids.

5. CORTICOSTEROIDS AND TRH

Thyroid hormones and glucocorticoids act synergistically to enhance biochemical, structural and functional lung maturity. A review of five randomized trials indicated that adding TRH to glucocorticoids significantly reduced the risk of RDS, severe RDS and chronic lung disease.[16] However, a recent large well-designed multi-centre randomized controlled trial[17] showed that the incidence of RDS and the need for assisted ventilation was greater among the infants of mothers treated with the TRH-glucocorticoid combination. Therefore, at present the use of TRH cannot be confidently recommended except in the context of a further

controlled randomized trial.

6. INDICATIONS FOR ANTENATAL CORTICOSTEROID THERAPY

Every effort should be made to initiate antenatal corticosteroid therapy in women between 24 and 36 weeks' gestation with any of the following :

Threatened preterm labour
Antepartum haemorrhage
Preterm rupture of the membranes
Any condition requiring elective preterm delivery

As pregnancy advances, the number of women that will have to be treated with corticosteroids to prevent a single case of RDS increases.

Antenatal education programmes or patient information leaflets should be considered to encourage early recognition of these conditions in an effort to ensure early presentation and commencement of treatment.

7. CONTRAINDICATIONS

Clinical suspicion of intrauterine infection and Tuberculosis

8. PRECAUTIONS

If betasympathomimetics are being used to delay delivery the volume of intravenous fluid administered should be kept to a minimum and chest pain, dyspnoea and cough should lead to immediate cessation of the beta-agonists. Women with suspected or confirmed rupture of the membranes should be closely observed for signs of chorioamnionitis. Women who have received repeated doses of treatment should have a glucose tolerance test. The extremely rare complication of adrenal insufficiency should be considered in the differential diagnosis of unexplained collapse in a woman or baby who has been exposed to repeated doses of antenatal corticosteroids.

9. DOSE AND ROUTE OF ADMINISTRATION

Two doses of betamethasone 12 mg given intramuscularly 24 hours apart or four doses of dexamethasone 6 mg given intramuscularly 12 hours apart.

This guideline was produced under the direction of the Scientific Advisory Committee of the Royal College of Obstetricians and Gynaecologists as an educational aid to obstetricians and gynaecologists. This guideline does not define a standard of care, nor is it intended to dictate an exclusive course of management. It presents recognised methods and techniques of clinical practice for consideration by obstetricians/gynaecologists for incorporation into their practices. Variations of practice taking into account the needs of the individual patient, resources and limitations unique to the institution or type of practice may be appropriate.

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