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CONTENTS:

Significance of Urine Kallikrein in the Prediction of Pregnancy-induced Hypertension

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Abstract : *A total of 158 urine kallikrein and creatinine levels were measured in 127 normotensive gravid women and 12 non-pregnant women and the kallikrein/creatinine ratio was evaluated to predict pregnancy induced hypertension. Urine kallikrein was examined using the MCA fluorescent method. Twenty-seven patients ultimately developed pregnancy-induced hypertension. In normotensive pregnant women, the urine kallikrein/creatinine ratio was significantly higher than that of non-pregnant women, especially during the first half of pregnancy. The mean urine kallikrein/creatinine ratio in patients destined to develop pregnancy-induced hypertension was lower than that of normotensive pregnant women at each gestational week and the difference was statistically significant at ≥ 32 weeks of gestation. When a kallikrein/creatinine ratio of less than 1.60 U/g creatinine was considered to represent a positive test, the predictive value of a positive test was high (71.4% at 32-35 weeks of gestation and 83.3% at ≥ 36 weeks of gestation), and there were no false nagative results at ≥32 weeks of gestation. The mean value of the latent period (interval between the time of a positive test result and the onset of pregnancy-induced hypertension) was 5.4 weeks. Evaluation of the urine kallikrein/creatinine ratio may be a useful screening tool in predicting the subsequent development of pregnancy-induced hypertension. (Thai J Obstet Gynaecol 1991;3:63-70.)*

Key words : pregnancy-induced hypertension, kallikrein

It has been reported that the urine content of kallikrein is decreased in patients with essential hypertension and glomerulonephritis, and is increased in patients with pheochromocytoma and primary aldosteronism[1-2]. Since the report by Elebute and Millo[3], there have been some studies of urinary kallikrein in normal pregnancy and in pregnancy-induced hypertension(PIH)[4-6]. In general, these studies found that urinary kallikrein activity in patients with PIH is significantly lower than in normal pregnant women. In this study, we used a fluorogenic substrate method[7] and estimated the amount of urinary kallikrein in uncomplicated pregnant

women at an early stage of pregnancy in an attempt to predict the onset of PIH.

Materials and Methods

A total of 127 pregnant women who were attending Kumamoto Municipal Women's Hospital for prenatal care and 12 volunteer non-pregnant women were entered in the study. Patients were selected randomly during gestation, and a total of 158 casual urine samples were collected. Women were excluded from the study if they had a history of chronic hypertension, diabetes or renal disease, or if at the time of entry into the study their blood pressure was ≥ 140/90 mmHg. They were also excluded if they had any evidence of proteinuria, as measured by the dipstick method. In all cases, gestation was estimated from the last menstrual period, and ultrasonic measurement of fetal crown-rump length was used early in pregnancy to confirm the gestational week. None of the subjects were taking medication, and their diets were unrestricted during the investigation.

After urine collection, the patients received routine prenatal care, and at the time of delivery their charts were reviewed for evidence of PIH. PIH was defined as a blood pressure of 140/90 mmHg or a rise of 30 mmHg in systolic pressure or 15 mmHg in diastolic pressure (measured twice, 6 hours apart at bed rest) with or without proteinuria and/or edema.

Urinary kallikrein was measured with a fluorogenic substrate method reported by Kato et al[7], using a fluorogenic peptide substrate, prolyl-phenyl-arginine-4-methyl coumaryl-7-amide (Pro-Phe-Arg-MCA). Using 20 μl of urine, the kallikrein activity was quantitatively assayed by incubation with a final concentration of 10^{-4} M Pro-Phe-Arg-MCA at 37°C for 90 minutes. The reaction was terminated by adding 20 μl of 10% acetic acid. The amount of MCA liberated was measured using a fluorescence spectrophotometer (Hitachi, Model MPE-2A). Intra- and interassay coefficients of variation were 2.4 and 4.1% respectively. We also measured the urine creatinine concentration using the alkaline pyruvate method (intra- and interassay coefficients of variation were 0.8 and 1.2% respectively) and the kallikrein/creatinine ratio was calculated for the prediction of PIH, since the concentration of kallikrein may largely depend upon the urinary volume in a casual urine sample.

Comparison of the groups was made using the unpaired Student's t-test for non-paired data. Values are expressed as standard error of the mean (SEM). Significance was set at the level of $p<0.05$.

Results

Twenty-seven (21.3%) of 127 pregnant women ultimately developed PIH and the remainders (100 patients) remained normotensive. Table 1 shows the clinical data of patients who developed PIH. The mean age of the pa-

Table 1 The clinical data of patients who developed PIH

No.	Name	Age	Parity	BBP	Max. BP	ΔSBP	ΔDBP	P	E	Onset	Del	BBW	UKK/UCr(Latent time)		
1	M. M.	24	0G0P	106/66	150/100	44	34	−	+	33	34	2,140	0.95 (2)	0.78 (1)	
2	Y. Y.	31	2G1P	90/60	120/90	30	30	+	−	38	38	3,120	0.47 (22)	0.51 (18)	
3	K. Y.	31	0G0P	102/50	122/72	20	22	−	−	37	40	3,240	2.63 (18)	2.32 (14)	
4	Y. M.	26	0G0P	110/62	128/80	18	18	−	−	39	39	3,660	1.06 (13)	1.47 (9)	
5	M. M.	27	1G1P	100/60	130/78	30	18	−	+	36	38	2,890	0.74 (12)	0.49 (4)	
6	A. A.	35	1G1P	110/70	140/98	30	28	+	−+	37	39	2,490	0.49 (3)		
7	Y. F.	24	0G0P	102/60	126/80	24	20	3+		37	40	3,090	1.84 (25)		
8	T. N.	38	3G3P	100/60	160/92	60	32	+	2+	34	37	2,300	1.54 (18)	0.82 (13)	1.55 (8)
9	S. T.	30	0G0P	104/60	120/90	16	30	+	−−	40	42	2,940	1.89 (28)	2.42 (28)	
10	E. T.	26	0G0P	134/74	130/90	−4	16	−	−	35	37	2,480	0.69 (8)	0.49 (4)	0.33 (0)
11	M. D.	36	0G0P	110/60	110/80	0	20	−	−	37	38	2,580	0.47 (1)		
12	T. T.	28	1G1P	104/64	128/80	24	16	−	−	39	40	3,080	0.27 (1)		
13	A. S.	27	0G0P	110/60	130/80	20	20	−	−	37	38	3,080	2.62 (22)		
14	F. S.	38	0G0P	110/70	140/88	30	18	−	−	38	40	3,000	0.56 (6)		
15	Y. S.	27	1G0P	120/60	122/80	2	20	−	2+	39	41	3,020	0.17 (1)		
16	M. K.	36	1G1P	110/64	130/80	20	16	−	−	39	39	2,850	1.56 (0)		
17	K. K.	31	0G0P	104/52	132/86	28	34	+	+	37	40	3,320	0.72 (14)		
18	S. K.	26	1G0P	110/60	120/88	2	28	−	+	39	40	3,740	0.72 (10)	1.07 (5)	0.72 (1)
19	N. G.	25	0G0P	112/60	124/76	12	16	−	+	38	41	3,390	4.2 (21)	2.2 (16)	2.8 (14)
20	Y. K.	25	0G0P	110/50	120/76	10	26	−	−	38	38	2,550	0.50 (2)		
21	M. K.	30	0G0P	130/78	140/96	−	−	+	−	36	38	2,840	1.55 (4)	0.79 (2)	
22	Y. K.	23	0G0P	110/68	112/82	2	14	+	−	40	42	3,270	1.11 (2)		
23	M. K.	26	1G0P	116/60	120/80	4	20	+	−+	38	39	3,390	1.1 (9)	1.6 (6)	0.6 (2)
24	K. E.	30	1G1P	130/80	118/92	−12	12	−	−	40	42	3,550	1.15 (2)		
25	K. I.	29	1G1P	120/66	122/88	2	22	+	−−	37	40	3,790	1.68 (24)		
26	U. I.	25	0G0P	108/54	120/80	12	26	−	−	37	39	2,770	2.2 (17)	1.9 (13)	2.1 (9)
27	H. I.	22	0G0P	102/62	136/80	34	18	−	+	38	38	3,020	2.18 (22)		

BBP; basal blood pressure, Max. BP; Maximum blood pressure, ΔDBP; Max. BP-BBP, P; proteinuria, E; edema, Onset; gestational week at the onset of PIH, Del; gestational week at termination, BBW; weight of newborn, UKK/UCr; urinary kallikrein/urinary creatinine, Latent time; the interval from documentation of a low ratio of UKK/UCr to the onset of PIH (weeks).

tients was 28.4 years (range 22 to 38 years). There were 19 primigravid and 8 multiparous patients. Blood pressure measured early in pregnancy was recorded as basal blood pressure (BBP). Maximum BP indicates the maximum blood pressure recorded during pregnancy. All but one patient (case No.8) had mild hypertension, and an elevated diastolic blood pressure was observed in 25 patients. Furthermore, the onset of PIH occurred near term, so small for gestational age babies were rare.

The mean values of urinary kallikrein in normotensive subjects at each gestational week and puerperium are shown in Fig.1. Early in pregnancy, the urinary kallikrein content was significantly higher than in non-pregnant women ($p<0.05$). In the latter half of pregnancy, the kallikrein content gradually decreased and was rather low in puerperium.

The mean value of the urinary kallikrein/creatinine ratio in normotensive subjects at each gestational week and puerperium is shown in Fig.2. The urinary kallikrein/creatinine ratio was biphasic over the course of preg-

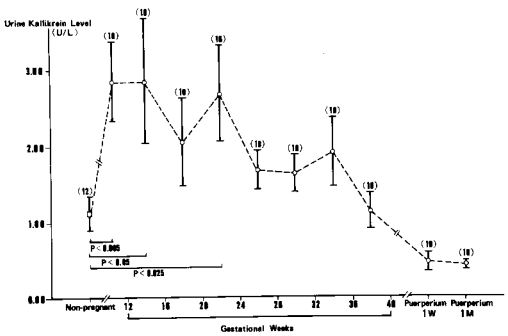

Fig. 1 Variation of urinary kallikrein levels during the course of normal pregnancy and puerperium. Values are expressed as Mean±SEM.

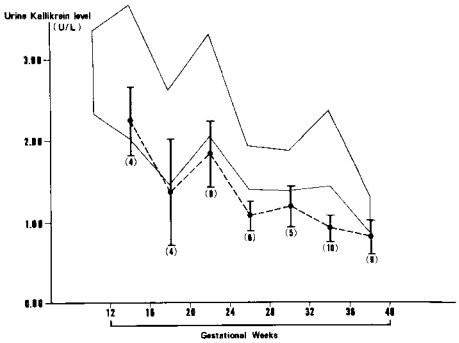

Fig. 3 Variation of urinary kallikrein before the onset of PIH in the women who finally developed PIH. Shaded area shows the range of normal pregnant women (Mean±SEM).

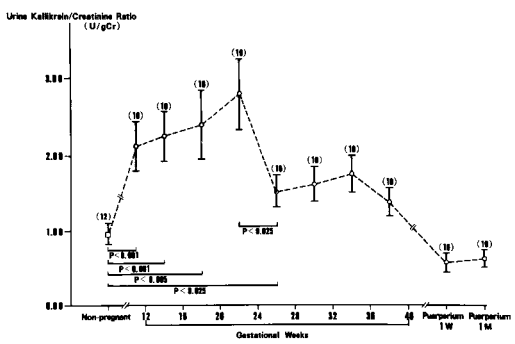

Fig. 2 Variation of the urinary kallikrein/creatinine ratio during the course of normal pregnancy and puerperium.

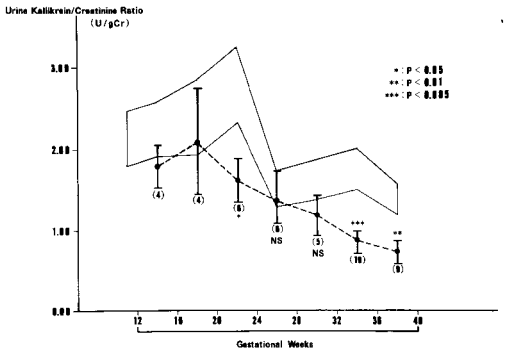

Fig. 4 Variation of the urinary kallikrein/creatinine ratio before the onset of PIH in the women who finally developed PIH. Shaded area shows the range of normal pregnant women (Mean±SEM).

nancy. Before 24 weeks of gestation, the ratios were significantly higher than in non-pregnant subjects. At around 24 weeks gestation, the ratio suddenly decreased to two thirds of that measured in the first half of pregnancy. This low value was maintained until near term.

The mean urinary kallikrein value in subjects who ultimately developed PIH is shown in Fig.3. The mean values in the PIH subjects were lower than those in normotensive subjects throughout pregnancy, but the differences were not significant.

The kallikrein/creatinine ratios

Table 2 Predictive parameters of PIH using the urinary kallikrein/creatinine ratio. PPV; Positive predictive value, NPV; negative predictive value

i) Cut-off value : 1.4 U/gCr

	sensitivity	specificity	P P V	N P V
GW 24～GW27	4/6 (66.7%)	5/10 (50.0%)	4/9 (44.4%)	5/7 (71.4%)
GW 28～GW 31	3/5 (60.0%)	6/10 (60.0%)	3/7 (42.9%)	6/8 (75.0%)
GW 32～GW 35	8/10 (80.0%)	6/10 (60.0%)	8/12 (66.7%)	6/8 (75.0%)
GW 36～	8/9 (88.9%)	6/10 (60.0%)	8/12 (66.7%)	6/7 (85.7%)

ii) Cut-off value : 1.6 U/gCr

	sensitivity	specificity	P P V	N P V
GW 24～GW27	4/6 (66.7%)	5/10 (50.0%)	4/9 (44.4%)	5/7 (71.4%)
GW 28～GW 31	4/5 (80.0%)	5/10 (50.0%)	4/9 (44.4%)	5/6 (83.3%)
GW 32～GW 35	10/10(100%)	6/10 (60.0%)	10/14(71.4%)	6/6 (100%)
GW 36～	9/9 (100%)	2/10 (20.0%)	9/17(52.9%)	2/2 (100%)

vi) Cut-off value : 1.8 U/gCr

	sensitivity	specificity	P P V	N P V
GW 24～GW27	4/6 (66.7%)	3/10 (30.0%)	4/11 (36.4%)	3/5 (60.0%)
GW 28～GW31	4/5 (80.0%)	3/10 (30.0%)	4/11 (36.4%)	3/4 (75.0%)
GW 32～GW35	10/10(100%)	6/10 (60.0%)	10/14(71.4%)	6/6 (100%)
GW 36～	9/9 (100%)	2/10 (20.0%)	9/17(52.9%)	2/2 (100%)

in subjects who ultimately developed PIH are shown in Fig.4. The mean values in the PIH subjects were again lower than those in the normotensive subjects throughout pregnancy, and the differences were significant after 32 weeks ($p<0.01$).

From these results, we selected some appropriate cut-off values and gestational weeks for the prediction of PIH. Three cut-off values and four gestational periods were chosen and the sensitivity, specificity, positive predictive values and negative predictive values were calculated for each condition (Table 2). In general, for any cut-off value, each predictive parameter became more reliable as gestation advanced. However, those patients who are at risk for PIH must be recognized as early as possible. When a kallikrein/creatinine value of less than 1.60 U/g creatinine was taken as a positive test result, the predictive value was high (71.4% at 32-35 weeks of gestation and 83.3% at 36 or more weeks of gestation). Furthermore, there were no false negative results at 32 or more weeks of gestation. Therefore, we chose a kallikrein/creatinine ratio of 1.60 U/g creatinine as the cut-off value and 32 weeks gestation as the time of examination.

In this study, we regarded a kallikrein/creatinine ratio of less than one standard error below the mean as abnormal. We also defined the period from which the abnormal results were obtained to the onset of PIH as the latent time. In PIH subjects, this latent time ranged from a few days to 28 weeks, and the mean was 5.4 weeks (Talbe 1).

Discussion

There are many reports concerning the prediction of PIH, including the angiotensin sensitivity test (AST)[8-10], roll over test[11], hand grip test and biochemical parameters such as serum uric acid and hematocrit[12-13]. Among these trials, the AST has been recognized as the most reliable method. However, it has not been confirmed as a practical screening test because of its difficulty in out patient clinics[10]. In this study, we were able to predict the onset of PIH with a high positive predictive and negative values, using a casual urine sample. It serves as a useful predictive parameter of PIH in prenatal care practice.

The most sensitive and specific method reported so far for the estimation of kallikrein in urine is the radioimmunoassay method[14]. However, it is necessary to prepare the antibody against bradykinin and the purified kininogen for this assay method. It is often difficult to obtain specific antibody in high titers. Against this, kallikrein contents estimated by the fluorogenic method were comparable to those obtained by radioimmunoassay[7]. Furthermore, this method is simple and the substrate is commercially available. From these points of view, it may be a useful screening tool in prenatal care, especially when dealing with a large number of urine samples.

Although a 24-hour urine collection was obtained from each patient in most of the previous reports[1-6], a casual urine sample was used for the estimation of kallikrein in this study. It has been reported that the concentration of kallikrein in a casual urine sample correlates to that of a 24-hour urine collection and daily excretion of urine kallikrein is independent of the 24-hour urine volume[15]. Based on these observations, we used the kallikrein/creatinine ratio in casual urine samples for prenatal screening.

Our results confirm the findings of Elebute and Millo[3] with regard to the increase in urinary kallikrein during the first half of pregnancy and the low excretion of kallikrein in late pregnancy. They also suggested that pregnant women with PIH had lower excretions of urinary kallikrein compared with that in normotensive pregnant women throughout gestation. However, a change in urinary kallikrein before the onset of PIH has not been confirmed. From our results, pregnant women destined to develop PIH had a significantly lower excretion of kallikrein compared with normotensive pregnant women at late pregnancy.

Kallikrein activity in urine is renal in origin and its excretion reflects the renal kallikrein-kinin system[16]. Although the interactions among the renal kallikrein-kinin system, renin-angiotensin-aldosterone system and vasoactive intrarenal prostaglandins have been reported[17], they are complex and so far only partly understood. Urinary kallikrein, however, correlates closely with renal blood flow[18], and the vasoconstriction induced by angiotensin II is suppressed by the renal kallikrein-kinin system[19]. From these observations, the decrease of urinary kallikrein in pregnant women may play an important role in the development of PIH.

In conclusion, urine kallikrein content increases in early pregnancy. It peaks in the first or second trimester and tapers off thereafter. In subjects who later develop PIH, the urinary kallikrein content is significantly lower than in normotensive pregnant women. Estimation of urinary kallikrein content is useful in predicting the onset of PIH. We propose that a kallikrein/creatinine ratio of 1.60 U/g creatinine by the fluorogenic method at 32 weeks gestation is a predictor of PIH.

Acknowledgement

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Fetal Biparietal Diameters in Normal Pregnant Southern Thai Women

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Abstract : *The Thai population has different anthropometric parameters from the Western standard. In order to utilize ultrasonographic data on fetal biparietal diameter (BPD) for prediction of fetal gestational age (GA) and growth monitoring, it is necessary to establish local standards. The objective of this study was to create our own normogram by ultrasonography and growth rate of the fetus at different gestational ages.*

A total of 901 normal pregnant women, residing in the Southern provinces of Thailand, between 12 to 40 weeks of pregnancy were measured for BPD by real-time ultrasonography. All of the subjects delivered normal fetuses. BPD curve versus gestational age showed two straight lines that joined at BPD 70 mm. Correspondingly, the growth rate of BPD is higher when GA or BPD is small and becomes lowest near term with a relatively rapid decline at the 28th week or BPD = 70 mm.

It is concluded that BPD can be easily measured by ultrasonography and helps in predicting the gestational age and growth rate of the fetus. (Thai J Obstet Gynaecol 1991;3:71-77.)

Key words: biparietal diameter, gestational age, ultrasonography

Reliable estimation of fetal gestational age (GA) remains an important problem in providing appropriate obstetric care since errors may result in significant morbidity and mortality[1,2]. Many reports have shown that a high percentage of pregnant women have uncertain menstrual data[3-5]. The accuracy of ultrasonography in determination of gestational age has been widely studied and its role in obstetrics has been increasing[6-8]. Because the fetal head grows continually and at a specific rate throughout

gestation, it has been observed that measurements of the fetal skull and, in particular, the biparietal diameter (BPD) allow for assessment of fetal age[7,8]. Formerly, we used information from Western countries to estimate GA from BPD[9-12]. However, because of the differences in race, and economic backgrounds, the formula may not be accurate for our local population[13]. The objective of this study was, therefore, to create our own normogram and study the BPD growth rate in our normal population.

Materials and Methods

From September 1986 to September 1990, a total of 901 pregnant women who attended the antenatal clinic at Songklanagarind Hospital, which is the only university hospital in Southern Thailand were studied. All these patients resided in Songkla and nearby provinces and had Thai nationality with GA between the 12th and 40th week. Most of the subjects were well educated and in the middle socioeconomic class. All subjects fulfilled the following criteria: pregnancies were single and without complications; no previous maternal disease; the date of onset of the last menstrual period was known and clinical estimations of maturity (e.g. growth of fundal height, quickening) agreed with the menstrual age calculated from that date; no hormonal contraceptives had been used for 6 months prior to pregnancy; regular menstruation for 3 months before pregnancy and ending with a full-term delivery and a full-term neonate defined by Dubowitz et al[14] with the birth weights between 2500 to 4000 g.

Hitachi Model EUB 200 and Toshiba Model Sonolayer SSA-250 with linear array (3.5 MHz) and curved linear array (3.75 MHz) transducers were used. The technique used for sonographic measurement was described by Hadlock et al[15]. Measurement for each fetus was taken three times within the same visit and the average was recorded and used for data analysis. The sample size was calculated by a formula described by Cohen[16]. The sample size in each week was at least 30 measurements, except in the 12th, 18th, 22nd, 23rd and 40th weeks.

At birth, the newborn was transferred immediately to the nursery and its birth weight taken with a beam balance with a confident precision of 10 g.

Results

The data were obtained from 901 subjects, each of whom contributed a single measurement.

Biparietal diameter and menstrual age

From Table 1, mean BPD increases steadily with GA. The standard deviations ranged between 1.78 and 4.44 mm. The coefficients of variation decrease with increasing GA, indicating higher precision in an older

Table 1 Mean fetal biparietal diameter values with 1 standard deviation for each week of pregnancy from 12 to 40 weeks

Weeks	Number of measurements	Mean biparietal diameter (mm)	1 SD	Coefficients of variation (%)
12	26	21.27	1.78	8.37
13	33	24.58	2.44	9.93
14	30	27.77	2.76	9.94
15	33	31.91	2.99	9.37
16	33	35.00	2389	8.26
17	30	38.57	3.20	8.30
18	19	42.58	3.70	8.69
19	31	43.55	3.60	8.27
20	30	47.47	3.03	6.38
21	30	49.70	3046	6.96
22	23	53.78	3.86	7.18
23	29	56.14	3.94	7.02
24	38	60.29	3.36	5.57
25	30	62.07	4.44	7.15
26	32	66.81	3.53	5.28
27	30	67.60	3.02	4.47
28	34	71.41	3.01	4.22
29	31	74.36	2.21	2.97
30	37	76.57	3.68	4.81
31	32	78.31	3.45	4.41
32	36	80.58	3.62	4.49
33	31	82.13	3.95	4381
34	32	84.75	2.90	3.42
35	32	86.97	3.10	3.56
36	35	88.46	3.28	3.71
37	34	89.74	3.28	3.66
38	33	91.55	3.09	3.38
39	34	92.74	2.74	2.95
40	23	93.17	2.73	2.93

fetus. The 10th and 90th percentile in each week are shown in Figure 1.

Figure 2 shows the scattered plot between biparietal diameter and gestational age. Visually, it can be observed that the distribution of the dots was in two straight lines joined approximately at BPD of 70 mm.

Figure 3 shows regression line when BPD is smaller than 70 mm. The resultant regression equation for the range was:

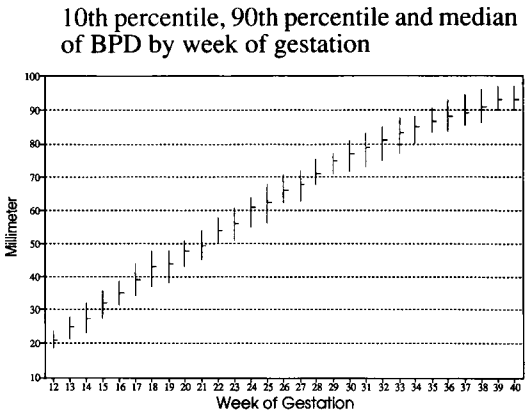

Fig. 1 Mean fetal biparietal diameter with upper and lower tolerance limits for each weeks of pregnancy from 12th to 40th week.

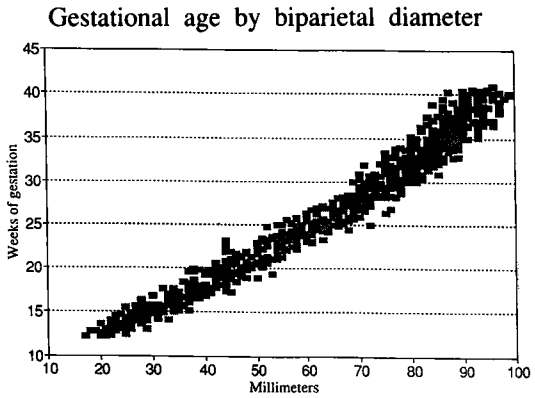

Fig. 2 Gestational age by biparietal diameter from 12th to 40th week.

GA (in week) = 0.311 BPD (in mm) + 5.851.....[1]

[r^2 = 0.95]

Similarly, Figure 4 shows regression line when BPD is equal to or greater than 70 mm. Here, the equation was:

GA (in week) = 0.458 BPD (in mm) - 4.189.....[2]

[r^2 = 0.83]

Growth rate at weekly intervals

The mean weekly increment of BPD is presented in relation to gestational age in Figure 5. The rate of increment steadily decreases from 3.25 mm per week at 18th week to 1.88 mm per week at 38th week. A relatively rapid drop in growth rate is observed near the 28th week which corresponds to BPD of 70 mm (Fig. 6).

Birth weight

The mean birth weight for the 901 infants was 3.18 Kg (SD = 0.36).

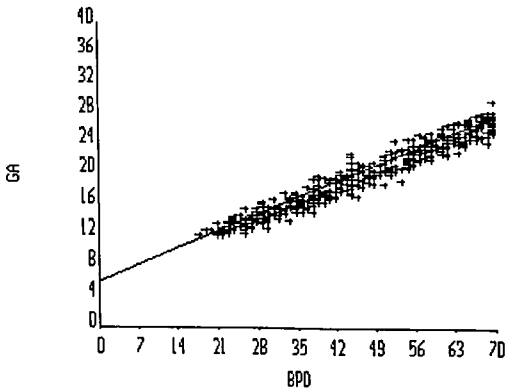

Fig. 3 Fetal biparietal diameter versus gestational age (when BPD <70 mm).

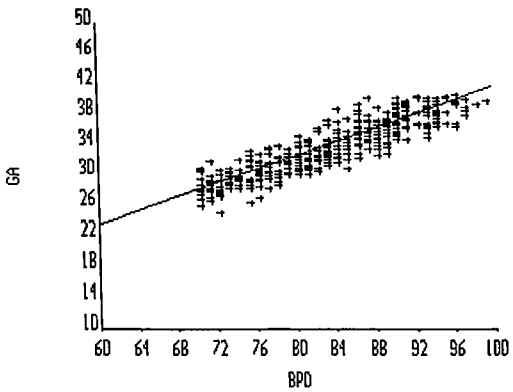

Fig. 4 Fetal biparietal diameter by gestational age(when BPD > 70 mm).

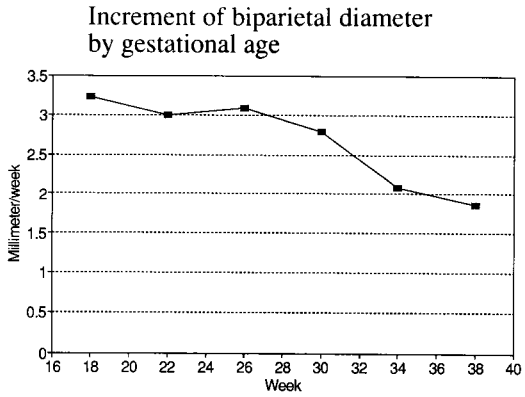

Fig. 5 Increment of biparietal diameter by gestational age from 16th week to 38th week.

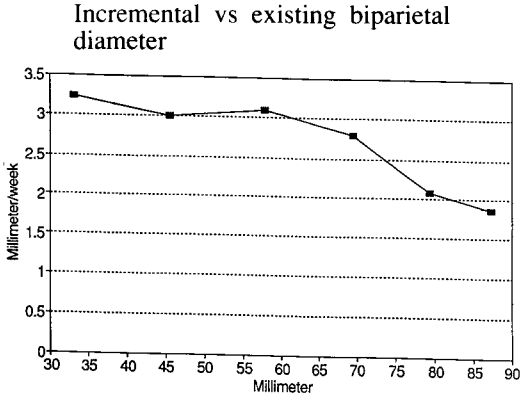

Fig. 6 Increment of biparietal diameter versus existing biparietal diameter.

Discussion

From the statistical calculation by Cohen[16] our sample size is enough to estimate the parameters in the population. The data produced here is considered to be accurate. However, as Watmough et al[17] stated, there appears to be no way of predicting those patients for whom errors in the ultrasonic reading will occur.

For a young fetus (small head), the greater error results from taking a scan at too widely separated distances, and sometimes an unclear landmark can be found, thus missing the true biparietal diameter. For a large head, the most important source of error is attributed to misdirection of the beam. For example, a beam directed 20 degrees away from the true BPD would give an overestimate which, for a head of BPD equal to 90 mm, will result in an error of 5 mm[12]. However, as all measurements were made by one person, three measurements within 2 mm range were required before a mean was taken, so that observational error would tend to be minimized. The mean birth weight of these newborns was also very close to that reported normal in this country[18]. Our data results are quite close to the others done in Western countries[9-12].

Our BPD versus gestational age curve can be separated into two linear regression lines. The coefficients of variation are high in the first trimester. Also in the third trimester, a high variation (low r^2) is noted. So, the precise estimate of fetal age by ultrasonography should be more accurate if done only in the second trimester. The rate of growth of BPD is higher when GA or BPD are small and becomes lowest near term with a relatively rapid decline at 28th week or 70 mm. Two consecutive measurements for determination of growth rate of fetal skull are crucial for differentiating a small-for-date fetus from a premature one. This is a great advan-

tage when monitoring a patient whose last menstrual period is not certain.

Ideally, growth rate must be calculated from the difference between two successive measurements on the same person, divided by the intervening time interval to the nearest date. Since we used cross-sectional data from 901 individuals, the computed growth rate was actually an interpolation and may have extra high variance. However, on the other hand, as this data came from several hundred subjects, each of whom contributed only a single point, the problem of over-representation of data from any subgroup was less probable. Therefore, generalizability of the results from such a study should be better.

This study presents the normogram of BPD versus GA, measured by realtime ultrasonography in a local population. It facilitates estimation of gestational age, and rate of growth. The benefit should be in high risk groups especially where there is uncertain date of last menstrual period and intrauterine growth retardation. Having access to exact data will help in further proper management.

Acknowledgement

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Ultrasound Fetal Femur Length in Normal Pregnant Northern Thai Women

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Abstract: *The measurement of fetal femur length (FL) in each week of pregnancy between the 14th and the 40th week was carried out at Maharaj Nakorn Chiang Mai Hospital form April 1989 to December 1990. A total of 1208 measurements were obtained from 435 patients, 203 patients had one measurement each and 1005 measurements were obtained from the remaining 232 patients. Growth of FL in the Thai populaion showed an asymptotic curve like that of Europeans or Americans but our values were significantly lower. The linear quadratic function was an accurate model describing the relation between femur length and menstrual age. Mean, standard deviation, 5th, 50th and 95th percentiles were calculated and predicted. FL for each menstrual week was also demonstrated. From the serial measurements performed, we found that the growth rate of the femur begins at 3.0 mm/week and slowly decreases to 1.3 mm/week toward 40 weeks of gestation. (Thai J Obstet Gynaecol 1991;3:79-83.)*

Key words: femur length, gestational age, pregnant Northern Thai women

Sonographic measurement of femur length as an indicator of menstrual age was first reported in 1981[1], and subsequently several investigators have used the fetal femur length as a predictor of menstrual age[2-4]. These studies have demonstrated that measurements of the fetal femur with ultrasound are very reproducible, probably because of the sharp bony margins. The primary objective of the initial studies was to detect dwarfism prenatally, and several reports have confirmed the usefulness of this data in this regard[5,6]. Many reports have indicated that the variability in predicting menstrual age from femur length is actually less than that of biparietal diameter (BPD). Unfortunately, no report on the growth of fetal femur length in pregnant Thai women has been conducted, and there

are some evidences indicating that the birth weight of Thai babies is somewhat lower than those of Western ones[7,8], therefore, we should accumulate our own data to have a standard value for further evaluation in Thai pregnancy.

Patients and Methods

The study included 435 pregnant Northern Thai women attending the antenatal clinic at Maharaj Nakorn Chiang Mai Hospital, Obstetrics and Gynaecology Department, Faculty of Medicine, Chiang Mai University, between 14-40 weeks. Each pregnancy was singleton, there was no medical, surgical or obstetrical complications during pregnancy. History of regular menstruation and exact date of last menstrual period was also noted, visit to antenatal clinic in first trimester and clinical estimation of gestational age agreed with menstrual age calculated from dates. Labour occured within 14 days of expected date of confinement, and Dubowitz's scores confirmed this age. Maternal height of all these patients fell within the normal range for Thai women.

All femur lenth measurements were performed by two perinatologists, well-trained for obstetric ultrasound, using linear-array real-time system with 3.5 MHz focused transducer (Aloka, Model SSD 630, 650). The technique originally described by O'Brein and co-workers[1] was used to align the transducer along the longest axis of the femur. The long axis of the fetus was identified first, and the transducer was then turned 90° to produce a cross-sectional image of the fetal trunk. The transducer was then moved down the fetus, maintaining this angle, to the fetal pelvis. Since the fetal femur is usually flexed, the transducer must be rotated 30° - 40° toward the fetal abdomen in order to visualize the long axis of the femur. Several femur length measurements were then made, and the longest measurement was considered optimal. Care must be taken to avoid tangential sections, which will foreshorten the femur, likewise, one must avoid including the ilium, ischium and distal femoral epiphyses, which will artificially lengthen the measurement. All measurements were made by using electronic calipers. The femur was determined in horizontal position in almost all cases, the maximum error in the lateral plane using the electronic calipers was 1%.

The perinatal sonographers did not know the menstrual age of the patients. Dubowitz's scores were assessed by only one pediatrician who had no any information about the obstetric data of the patients.

Results

A total of 1208 measurements of the fetal FL from the 14th to 40th week were taken in 435 patients, 203 patients had one measurement each, and the remaining 232 had serial measurements at least twice. The mean of FL for each gestational week and 2SD were calculated and the val-

Table 1 Mean fetal FL with 2SD, 5th, 50th and 95th percentile for GA

GA weeks	No.of exam.	Mean (cm)	2 SD (cm)	5th percentile	50th percentile	95th percentile
14	34	1.34	0.32	1.0	1.3	1.5
15	40	1.49	0.40	1.2	1.4	1.8
16	36	1.64	0.52	1.3	1.6	2.0
17	44	2.06	0.54	1.4	2.0	2.5
18	41	2.33	0.46	1.8	2.3	2.6
19	45	2.64	0.54	2.1	2.6	3.1
20	47	2.82	0.44	2.4	2.8	3.1
21	49	3.22	0.52	2.6	3.2	3.6
22	41	3.38	0.60	2.8	3.4	3.8
23	40	3.71	0.46	3.2	3.7	4.0
24	43	3.94	0.54	3.4	3.9	4.2
25	41	4.19	0.46	3.7	4.1	4.5
26	42	4.36	0.62	3.7	4.3	4.8
27	45	4.65	0.42	4.2	4.6	4.9
28	45	4.88	0.66	4.4	4.8	5.5
29	48	5.17	0.66	4.6	5.1	5.9
30	47	5.26	0.64	4.8	5.2	5.8
31	59	5.58	0.66	5.0	5.5	6.1
32	49	5.70	0.60	5.1	5.7	6.1
33	50	5.88	0.66	5.4	5.8	6.4
34	48	6.05	0.54	5.5	6.0	6.4
35	48	6.32	0.74	5.6	6.3	6.9
36	48	6.37	0.50	5.8	6.4	6.8
37	55	6.52	0.66	5.9	6.5	7.1
38	49	6.67	0.60	6.1	6.6	7.2
39	41	6.84	0.60	6.2	6.9	7.2
40	40	6.91	0.58	6.4	6.9	7.3

ues are shown in Table1 and Figure 1. There was progressive linear increase from the first trimester towards term. In addition, 5th, 50th and 95th percentiles were also calculated, as shown in Table 1 and Figure 2. The linear quadratic function was an accurate model for describing the relation between femur length and gestational week (r = .98). The correlation between femur length and gestational age was formulated, FL = -4.09575 + 0.42633(GA) - 0.00377304(GA^2) [GA = weeks of gestational age]. Comparison of mean FL (mm) for each gestational week between O'Brein's and this study was analyzed and showed that the femur length of pregnant Northern Thai women in this series was significantly lower than that in the Western series in almost all gestational age. The growth rate of the femur from serial measurement data has also been calculated, and found

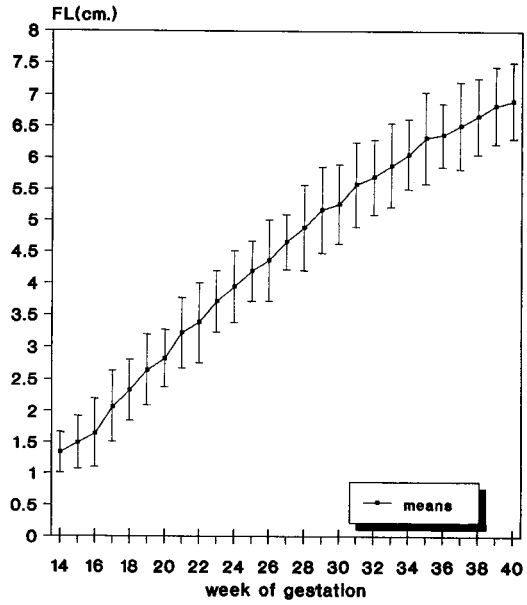

Fig. 1 Femur length and gestational age in normal pregnant Northern Thai women.

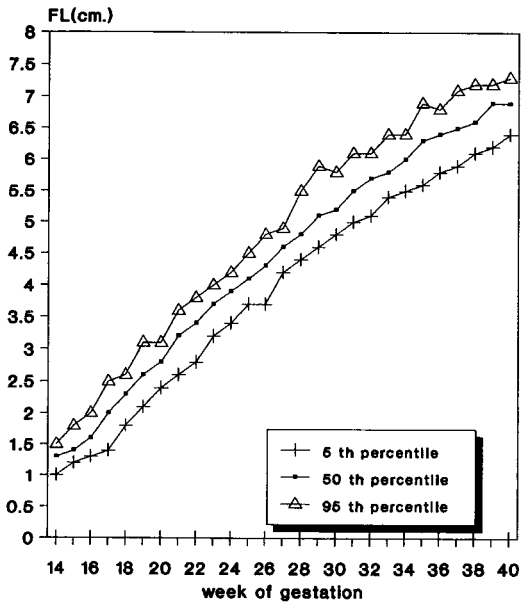

Fig. 2 Percentile chart of FL in normal pregnant Northern Thai women.

that growth rate begins at 3.0 mm/week and slowly decreases to 1.3 mm/week at 40th week of gestation.

Discussion

High-resolution real-time ultrasound now makes it possible to evaluate many physical parameters of the fetus. Fetal femur length measured by ultrasound provides another reliable parameter for the estimation of gestational age of the fetus. The value can serve as an alternative to the BPD measurement. Ideally, it will be used as an adjunct to BPD measurement, providing a more accurate evaluation.

Some investigators found that there may be race variations in true femur lengths that should be taken into account for prediction of gestational age[9]. For example, the value currently used to estimate menstrual age from femur length is based predominantly on the white Anglo-Saxon population. Hayashi[9] has demonstrated that on average, fetuses of Latin-American origin have femur lengths that are somewhat shorter than those in the white Anglo-Saxon population. Pathologically, studies in adults indicate the posibility that some segments of the black population may have femurs that are on average longer than those observed in the White Anglo-Saxon population[9]. In comparison, mean femur length value of each gestational week in pregnant women from a Northern Thai population was quite different from those in Western reports[1,2,10-12]. The femur length values in our series agree with Hayashi's observation[9], and are consistent with the fact that average birth

weight of a Thai baby is lower than those of the European or the American ones[7,8]. The difference of mean femur length values in our study and Western studies is most likely due to the racial factor.

Ultrasound fetal femur length values in the present study may be more appropriate for pregnant Northern Thai women than employing the European or American ones. In addition to estimating gestational age, fetal femur length may be useful as an adjunct in the diagnosis of some abnormalities, i.e. short limb syndrome or osteogenesis imperfecta.

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Investigation of Serum Lipid Levels During Mid-trimester Prostaglandin Induced Abortion

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Abstract : *Concentrations of serum lipids rise during the progression of gestation, reaching the peak at the end of pregnancy. Although these changes are reversible, the possible influence of elevated serum lipids on atherosclerotic and ischemic cardiovascular disease as well as biliary tract lithiasis development is still unresolved. The aim of this study was to investigate concentrations of serum lipids during mid-trimester pregnancy abortion induced by $PGF_{2\alpha}$. Blood samples were taken four times: from the cubital vein of 30 women, aged from 15-40, admitted for mid-trimester pregnancy abortion I. before intramuscular application of $PGF_{2\alpha}$ (control) and II. three hours after, in the active phase of abortion (III) and twelve hours after the curettage (IV). Quantitative assay was used for determination of total cholesterol, triglycerides, phospholipids, LDL and HDL, while apolipoprotein A1 and apolipoprotein B were determined by immunochemical methods. Three hours after prostaglandin administration levels of triglycerides, LDL and apolipoprotein B were higher, while other lipids were lower, compared to the control group. During the abortion's active phase all lipid concentrations were higher, compared to the control but only HDL lipoproteins were lower. Twelve hours after curettage serum lipid concentrations had decreased, reaching control levels; only serum triglycerides were higher. Apolipoprotein A1 and apolipoprotein B were supposed to have a protective role against atherosclerotic process and biliary tract diseases development. (Thai J Obstet Gynaecol 1991;3:85-88.)*

Key words: mid-trimester pregnancy abortion, prostaglandins, serum lipid levels

According to the literature about half of pregnancies are terminated by abortion despite a bulk of investigation in medical sciences. During only one year about 35-55 million abortions are performed in the world while 84000 of women die due to pregnancy interruption complications[1]. It is also well known that mid-trimester pregnancy abortion has a twenty times higher morbidity and mortality risk compared to a first trimester abortion[2].

Elevation of serum lipid levels during pregnancy is progressive and reversible[3]. Previous investigations

confirmed that serum lipid concentrations rise during mid-trimester pregnancy abortion induced by hypertonic saline solution and proposed that their investigation may be of use for the study of arterosclerotic and ischemic cardiovascular disease as well as for biliary tract lithiasis development[4]. So, the aim of this research was to investigate serum lipid levels during mid-trimester pregnancy abortion induced by $PGF_{2\alpha}$ and the possible role in pathogenesis of those diseases.

Materials and Methods

The study comprised of 30 women, aged from 15-40, without any endocrine or metabolic disorders admitted for mid-trimester pregnancy abortion for social reasons.

After routine investigations on admission, gestational age was assessed by gynaecological and ultrasonographic examinations. Peripheral blood samples from cubital vein were taken four times: before intramuscular application of $PGF_{2\alpha}$ (I) (control group) and three hours after that (II), in the active phase of abortion (III) and twelve hours after the placental expulsion and curettage (IV). The levels of total cholesterol, triglycerides, phospholipids, LDL and HDL were investigated by quantitative assays while concentrations of apolipoprotein A1 and apolipoprotein B were determined by immunochemical methods. Obtained data were tested by variance analysis for attributive parameters.

Results

Serum lipid levels during mid-trimester pregnancy abortion induced by $PGF_{2\alpha}$ are shown in Figure 1.

	Before PG application	3h after PG application	During abortion's active phase	12 hours post abortion
Cholesterol (mmol/l)	6.50 +/- 1.09	6.28 +/- 0.72	7.07+/- 1.31	6.85 +/- 1.35
Triglycerides (mmol/l)	2.04 +/- 0.70	2.59 +/- 0.72	2.35 +/- 0.43	2.57 +/- 0.62
Phospholipids (mmol/l)	3.26 +/- 0.32	3.21 +/- 0.24	3.56 +/- 0.37	3.20 +/- 0.24
LDL (mmol/l)	3.57 +/- 1.06	3.83 +/- 0.74	4.38 +/- 1.25	4.30 +/- 0.99
HDL (mmol/l)	1.81 +/- 0.55	1.26 +/- 0.21	1.62 +/- 0.44	1.40 +/- 0.63
Apolipoprotein A1 (g/l)	1.92 +/- 0.17	1.70 +/- 0.19	1.96 +/- 0.35	1.56 +/- 0.31
Apolipoprotein B (g/l)	1.51 +/- 0.35	1.55 +/- 0.31	1.70 +/- 0.37	1.60 +/- 0.47

Fig. 1 Serum lipid levels during mid-trimester pregnancy abortion induced by $PGF_{2\alpha}$.

Concentrations of triglycerides, LDL and apolipoprotein B were slightly higher three hours after intramuscular application of $PGF_{2\alpha}$ compared to the controls, while levels of other investigated lipids were lower, but not significantly ($p > 0.05$).

During the active phase of abortion serum lipid levels were higher compared to the controls, while HDL lipoproteins were significantly lower ($p < 0.05$).

Twelve hours after the placental expulsion and curettage, serum lipids were decreased compared to the previous respective sample concentrations; only serum triglycerides were higher, but not significantly ($p > 0.05$). Serum lipid levels in this postabortive phase were comparable to those before the prostaglandin application.

Discussion

There is a relatively small amount of investigations of serum lipid levels during mid-trimester pregnancy abortion. Changes of serum lipids during the abortion active phase is due to the stress effect on pituitary-adrenal axis, and elevation of ACTH and cortisol concentration in serum[5].

In healthy persons, during pregnancy, there is a steady and significant increase of serum cholesterol levels and LDL lipoproteins, but also a decrease of HDL lipids. Maximal serum lipid concentrations are registered between 33-36 weeks of gestation[3]. Jimenez et al[6] found that HDL is in negative correlation with cholesterol and triglycerides during the second half of gestation. Our investigation confirmed significantly lower HDL levels after application of $PGF_{2\alpha}$. This finding may indicate a protective role of HDL in atherosclerosis and ischemic myocardial disease development, but biochemistry of this protective mechanism is still unclear[7]. Steady increase of serum cholesterol levels during gestation are due to progressive concentration rise of LDL. Composition of these lipoproteins is changed and manifested in decreased ratio of cholesterol and apolipoprotein B[8]. This finding is not in correlation with estrogen and progesterone levels rise. Phospholipids are very important for metabolism of arachidonic acid, as precursors of prostaglandins, prostacyclins and thromboxans[9]. Increase of serum phospholipid concentrations that we found is due to degenerative and necrotic changes in feto-placental compartment. Apolipoprotein A1, constitutive part of HDL and chilomicrones, stimulates hepatic lipoprotein lipase and is very important in cholesterol and HDL metabolism[10]. Clinical value of apolipoprotein A1 determination is still unresolved, but we found that it's concentrations were lower after the abortion procedure was completed compared to those before the prostaglandin application. We propose that it has, besides a HDL, a protective role in atherosclerosis and ischemic myocardial disease as well as biliary tract lithiasis development. Zannis and Brseslow[11] regarded that concentrations rise of apolipoprotein B

is due to LDL binding to their specific receptors. The most recent studies have indicated that elevated apolipoprotein B is more important for atherosclerotic change development in blood vessels than decreased concentration of HDL[7].

In conclusion one can say that serum lipid levels require further investigation for elucidation of pathophysiological changes during pregnancy as well as for cardiovascular and biliary tract diseases commencement.

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Bacteriologic Study of Donor Semen

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Abstract : *Semen samples from 63 donors were bacteriologically studied to determine the bacterial isolates, their influence on sperm quality and the recipients. Two of the 63 samples had no bacterial growth, 61 cultures yielded one or more aerobic organisms, and 42 yielded anaerobic organisms. The 4 common aerobes were S. epidermidis (50.8%), Streptococcus group D enterococci (30.2%), S. viridans (25.4%), and Diphtheroides (25.4%). These organisms had no influence on semen parameters. The anaerobic organisms, which included Peptostreptococcus (60.3%), nonsporing gram-positive rods (17.5%), Bacteroides (14.3%), were related to sperm concentration and motility. All recipients had no complications after donor insemination. Four of the 21 recipients became pregnant, and the semen inseminated to these women contained from 1 to 6 types of organisms. The routine aerobic and anaerobic bacterial culture of selected donor semen seem not be necessary. (Thai J Obstet Gynaecol 1991;3:89-94.)*

Key words: bacteriologic study, donor semen

Donor insemination is widely practiced for treatment of male infertility. It has been shown previously that semen used for artificial insemination may be contaminated with a variety of microorganisms, some of which are potentially pathogens[1,2]. Despite this phenomenon the symptomatic rate of pelvic infection following insemination was very low[3]. Furthermore, semen quality seem not related to aerobic or anaerobic bacterial cultures of semen[4].

The purpose of the present study was to determine the bacterial isolates in donor semens and the influence of such isolates on sperm quality.

Materials and Methods

Sixty-three semen samples were obtained from healthy adult males of 21 to 36 years of age and were randomly selected from those attending the artificial insemination donor program of Infertility Unit, Department of Obstetrics and Gynaecology, Siriraj Hospital, from March 1988 to June 1989. Before collecting the specimens, all donors were instructed to wash their hands and the glans penis with soap and water thoroughly, or to clean the glans penis with a sterile cloth. The semen was collected by masturbation - ejaculation

into a sterile wide-mouthed glass jar and was divided into three portions, 0.5-0.8 ml for bacterial culture, 0.5 ml for analysis and the rest for insemination. Culture for aerobic and anaerobic organisms was carried out, within 1 hour after collection of specimen, by conventional method. Semen analysis was performed by the standard technique described by WHO[5].

The ovulatory day of the cycle was determined by BBT and cervical mucus. The donor semen, 0.3 ml, was inseminated into the cervical canal while the rest was deposited in the posterior fornix.

Statistical analysis of the results was performed employing the Student's unpaired t-test or ANOVA as appropriate.

Results

Twenty one women with normal infertile work-up, whose husbands were azoospermic, were included in the study. Their ages ranged from 21 to 36 with the infertility period between $1\frac{1}{2}$ to 10 years. Of the 63 cultures, no organism was found in only 2 (3.2%). Among the remaining 61 samples, 1 to 4 organisms were isolated. Only one specimen harboured 7 different organisms (Fig. A).

The aerobic organisms isolated and the sperm characteristics of the ejaculates are shown in Table 1. To determine whether semen quality was affected by certain aerobic organisms, it was found that there was no association between a rich culture of aerobic organisms and sperm concentration and motility ($p > 0.05$).

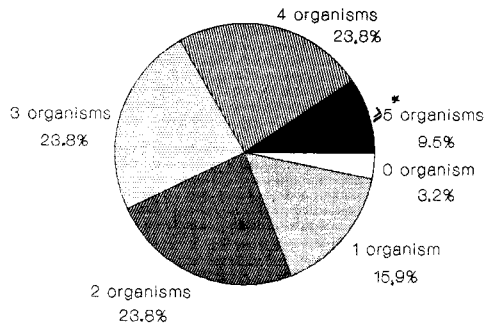

Fig. A Number of organisms isolated from 63 donor semens.
* maximum = 7.

Table 2 shows the anaerobic organisms isolated and their correlation to sperm concentration and motility. When comparing 21 ejaculates without anaerobic organsims with 42 ejaculates with anaerobic organisms, it was found that the sperm concentration and motility was significantly better among those with than those without growth of organisms ($p<0.05$).

Four women (19.0%) became pregnant. The bacterial growths in the semens that inseminated these women are shown in Table 3. All semens harboured 1 to 6 varieties of organisms.

None of the women in this study had either fever, abnormal vaginal discharge, or lower abdominal pain after insemination.

Discussion

Despite several species of organism, as many as 7, were identified from the same ejaculates. These organisms could account for the con-

Table 1 Aerobic bacteria and semen characteristics

Aerobic bacteria	No. of samples	Sperm count (x 10^6 / ml) ($\bar{x} \pm SD$)	Sperm motility (%) ($\bar{x} \pm SD$)
S. epidermidis	32	71.4 ± 56.3	74.6 ± 13.4
S. group D enterococci	19	67.6 ± 56.0	76.2 ± 13.6
S. viridans	16	77.6 ± 65.6	68.4 ± 17.6
Diphtheroides	16	65.4 ± 35.9	71.1 ± 12.1
Nonfermentative gram - negative rods	7	73.8 ± 66.0	79.8 ± 9.5
B. subtitis	5	55.0 ± 44.3	78.0 ± 8.8
Hemolytic streptococcus non group A,B,D.	4	47.0 ± 30.9	73.5 ± 11.1
P. mirabilis	4	64.7 ± 35.6	76.0 ± 4.1
H. parainfluenza	2	50.5 ± 0.7	66.5 ± 33.2
K. pneumoniae	2	41.0 ± 1.4	73.5 ± 13.4
P. morgagni	1	63	65
E. coli	1	48	72
S. aureus	1	52	80
S. saprophyticus	1	65	89
A. hydrophila	1	44	88
Corynebacterium species	1	63	85
Micrococcus species	1	169	84
No growth	3	56.3 ± 44.7	65.0 ± 11.1

Table 2 Anaerobic bacteria and semen characteristics

Anaerobic bacteria	No. of samples	Sperm count (x 10^6 / ml) ($\bar{x} \pm SD$)	Sperm motility (%) ($\bar{x} \pm SD$)
Peptostreptococcus	38	63.4 ± 39.0	71.4 ± 14.4
Nonsporing gram - positive rods	11	63.1 ± 54.3	68.8 ± 11.6
Bacteroides	9	56.8 ± 20.8	69.4 ± 18.7
Fusobacterium	2	26.5 ± 19.1	70.5 ± 13.4
V. parvula	1	111	75
No growth	21	91.9 ± 64.3	80.2 ± 9.7

Table 3 Bacterial growths in semen inseminated to 4 pregnant recipients

Pregnancy	Aerobic bacteria	Anaerobic bacteria
1	S. epidermidis S. viridans Diphtheroides	Peptostreptococcus Bacteroides Non-sporing gram positive rods
2	S. epidermidis S. viridans S. group D enterococci	Peptostreptococcus
3	S. epidermidis	
4	S. epidermidis S. viridans Non-fermentative gram - negative rods	Peptostreptococcus

tamination of bacterial flora of the skin or distal urethra. However, these findings are similar to those previously reported[1,2,4,6-9].

With regard to the relationship between the presence of organisms in the semen and the sperm quality, it seems that aerobic bacteria does not play an important role in reducing sperm concentration and sperm motility, but it seems to be reduced among ejaculates who harbour anaerobic bacteria. These figures are slightly different from other studies which reported no association between sperm characteristics and the presence of aerobes or anaerobes isolated from the semen[4,10,11]. Several authors reported that U. urealyticum was found in significant numbers among the semen of poor quality[4,12,13]. On the other hand, there is no relationship between abnormal semen parameters and the presence of U. urealyticum as reported by others[14,15]. This organism was not of interest in this series.

Despite potentially pathogenic organisms, such as hemolytic streptococci, Klebsiella, E.coli and S. aureus were identified in this study but none of the patients developed clinical infection after insemination. It is believed that cervical mucus may play a role of the effective mechanical and immunologic barrier thereby preventing the ascending infection to the upper female genital tract. However, it has been demonstrated *in vitro* that bacteria can be carried by sperm through a cervical mucus column[16]. Stone et al[17] reported that after artificial insemination, microorganisms were discovered from the peritoneal fluid.

The influence of the presence of bacteria in the semen on the fertilizing capacity of sperm is not known. Since all pregnancies from artificial

insemination of the semen harboured 1 to 6 different organisms, further study should be carried out to disclose this event.

As a result of this study, although a conclusion cannot be made, it is felt that routine culture of aerobic and anaerobic bacteria of selected donor semen is not necessary. However, one should practice restricted precautions in order to prevent a transfer of a sexually transmitted disease or pathogenic bacteria by means of ejaculate used for donor insemination.

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Two-year Experience with Gamete Intrafallopian Transfer (GIFT) at Maharaj Nakorn Chiang Mai Hospital

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Abstract : *This article describes twenty patients who underwent gamete intrafallopain transfer (GIFT) at Maharaj Nakorn Chiang Mai Hospital during a two-year period. The details of this technique are described. The pregnancy rate, as defined by a rising of ß-hCG titer on two occasions at least 2 days apart, was 31.8% per treatment cycle, but the clinical pregnancy rate was only 27.4% per cycle. This technique could become an alternative to in vitro fertilization and embryo transfer (IVF & ET) in infertile women who have at least one patent fallopian tube. (Thai J Obstet Gynaecol 1991;3:95-102.)*

Key words : gamete intrafallopian transfer (GIFT), pregnancy rate, infertility

Recently many new reproductive techniques have been introduced with varying success rates to help infertile couples achieve pregnancies. These include superovulation with human menopausal gonadotropins (hMG)[1], intrauterine insemination (IUI)[2], intratubal insemination (ITI)[3], direct intraperitoneal insemination (DIPI)[4], peritoneal oocyte and sperm transfer (POST)[5], gamete intrafallopian transfer (GIFT)[6], zygote intrafallopian transfer (ZIFT)[7] and in vitro fertilization/embryo transfer (IVF/ET)[8]. In this report, we present our experience with the GIFT technique over a two-year period from October 1, 1989 to August 31, 1991.

Materials and Methods

Patient selection

All couples underwent complete basic infertility investigations, which included at least two semen

analyses, confirmation of ovulation with endometrial biopsy or mid-luteal serum porgesterone assays, post-coital tests and laparoscopic tubal patency tests. Patients with endometriosis were treated with expectant treatment and/or a course of danazol with or without surgery depending on the extent of the lesions. Those with cervical factor infertility were treated with intrauterine insemination with or without clomiphene citrate for ovulation induction. Oligospermic males, defined as a sperm count of less than 20 X 10^6/ml, were treated with mesterolone acetate for 4-6 months, with or without split ejaculate insemination. Couples with unexplained infertility were empirically treated with clomiphene citrate superovulation for 4-6 months. Prior to being considered for GIFT, each couple had exhausted all conventional treatment for their infertility problems as stated above. Only those with at least one patent fallopian tube were apporached for informed consent after detailed discussion of the GIFT procedure.

Controlled ovarian hyperstimulation

In our clinic, both long and short stimulation protocols, as previously described by others[9], are in use. The decision to place patients on either the long or the short protocol was made at the discretion of the attending physicians and not randomly. In brief buserelin acetate (Suprefact®, Hoechst) was administered intranasally at a dose of 100 μg, six times per day, starting in the mid-luteal phase of the preceding cycle in the long protocol, or on the first day of the current cycle in the short protocol. 2-4 ampules of human menopausal gonadotropins (hMG, Pergonal®, Serono) were given intramuscularly, according to the patient's age and previous response, from cycle day 3 onward. Pelvic sonogram was done on the first day of the cycle as baseline, and then on a daily basis from day 8 of the cycle to monitor follicular growth. Daily measurement of serum estradiol by radioimmunoassay was done on day 6 of the cycle. Human chorionic gonadotropin (hCG, Profasi®, Serono) 10000 IU was given intramuscularly when ultrasound demonstrated two or more ovarian follicles exceeding 17 mm in diameter an the estradiol level reached 300 pg/ml per dominant follicle. Buserelin was stopped on the day of hCG injection and oocyte retrieval via laparoscopy, minilaparotomy or vaginal aspiration under ultrasound guidance, was scheduled 34-36 hours later.

Sperm preparation

Male partners were requested to produce semen samples approximately 2.5 hours before oocyte retrieval. Semen was allowed to liquefy for 30-45 minutes before analysis and the results recorded on a standard form. Semen was gently mixed with Ham's F-10 (GIBCO, New York) at a ratio of 1:2 (vol/vol) and centrifuged for 10 minutes at 300 g. The superna-

tant was discarded and the sperm pellet resuspended in 1 ml of medium. After a second wash, 0.5 ml of medium was layered onto the loosened pellet and motile sperm were allowed to swim up for 45 minutes in a 5% CO_2 incubator at 37° C. Sperm concentration in the supernate was reassessed and adjusted to 100000/2.5 µl.

Oocyte preparation

Fluid from follicular puncture was examined carefully to recover oocytes under a dissecting microscope. Each harvested oocyte was placed in 1 ml of Ham's F-10 supplemented with 10% of the patient's serum in a 4-well plate, and kept in an incubator under 5% CO_2 in air at 37° C until transfer. After completion of oocyte retrieval, mature oocytes were selected and placed in a drop of Ham's F-10 supplemented with 50% of the patient's serum.

Tubal gamete transfer

Gamete transfer catheter (William A. Cook, Australia), with 1-ml tuberculin syringe attached at one end, was rinsed twice with Ham's F-10 supplemented with 50% of the paient's serum. Sperm and oocytes were loaded into the catheter in the following sequence: 10 µl of medium, an air space of 5 µl, 25 µl of sperm preparation containing 100000 sperm, an air space of 5 µl, one or more mature oocytes in 25 µl of medium, an air space of 5 µl and finally 10 µl of medium. The catheter was then introduced into the fimbriated end of the fallopian tube to a distance of 1.5-2 cm under laparoscopic control or under direct visualization in case of minilaparotomy. The content was gently expelled into the ampullary region of the tube. The catheter was then slowly withdrawn and returned to the laboratory in order to ensure that gametes were expelled. A similar procedure was repeated with the contralateral tube.

Luteal phase support

All patients received hCG (Pregnyl®, Organon) 1500 IU intramuscularly on the day of gamete transfer and on days 3, 6 and 9 after the transfer.

Diagnosis of pregnancy

Pregnancy was diagnosed on day 14 post-GIFT when the level of serum ß - hCG was greater than 25 mIU/ml, followed by a higher level in a subsequent assay 2 days later (Biochemical pregnancy). Clinical pregnancy was diagnosed when a gestational sac was visualized under vaginal ultrasound, which should be clearly seen from day 35 post-GIFT.

Results

Twenty-six infertile couples were enrolled for 28 cycles of GIFT. There were two patients who underwent the GIFT procedure twice. Of

these, six cycles in six patients were cancelled before oocyte retrieval; five because of poor ovarian response to hMG and one because of decreasing estradiol level before oocyte pick-up.

The average age of the twenty remaining patients was 34.9 years (ranged 29-42 years). All had fallopian tube patency on both sides. Fourteen of them had primary and six had secondary infertility, for an average duration of 7.8 years (ranged 2-14 years). Their infertility diagnoses are shown in Table 1. Two couples had both endometriosis and male factor infertility, one had both endometriosis and cervical factor infertility. Long and short ovulation induction protocols were used in equal number of cycles. On the average 25.8 ampoules of hMG were needed in the long protocol versus only 12.3 ampoules in the short protocol. However, there was no significant difference in the number of oocytes harvested. Pre-washed sperm motility increased from 43.1% to 77.2% after washing. An average of 4.7 oocytes were transferred per cycle (ranged 2-10). The gametes were transferred into both fallopian tubes in 10 cycles, resulting in 4 pregnancies. In 12 cycles, the gametes were deposited into only one tube, resulting in 3 pregnancies. The overall pregnancy rate was 31.8%, but clinical pregnancy rate was only 27.3% (Table 2).

Five pregnancies occurred in patients with endometriosis and one each in patients with cervical factor and unexplained infertility. There was no pregnancy in the six couples who had male factor infertility. The success of this procedure when stratified by the women's ages and by the number of oocytes transferred are shown in Tables 3 and 4.

Table 1 Infertility diagnosis

Diangnosis	Number
Endometriosis	13
Male factor	6
Carvical factor	2
Unexplained infertility	2

Table 2 Outcome of GIFT cycles

	Long protocol	Short protocol
Number of cycles	11	11
Number of hMG used (ampoules)	25.8	12.3
Number of oocytes harvested	5.3 (2-10)	4.7 (2-8)
Pregnancy	5	2
Biochemical	0	1
Clinical	5	1

Table 3 Pregnancy by women's ages

Age (years)	Number of patients	Number of pregnancy
<30	1	0
31-35	11	4
36-40	7	3
>40	1	0

Table 4 Pregnancy by the number of oocytes transferred

No. of oocytes transferred	No. of patients	No. of pregnancy
1-2	3	1
3-4	10	2
5-6	7	3
7 or more	2	1

Of the six clinical pregnancies there were two sets of twins, one of which aborted at 11 weeks pregnancy and the other is still on-going. Of the four remaining pregnancies, one aborted at 14 weeks, one was delivered preterm at 36 weeks (male fetus 2460 g), and two went on to term with uneventful deliveries.

The operating time for GIFT procedure was 74.5 minutes (ranged 46-95 minutes) when performed through minilaparotomy and only 25 minutes when done via laparoscopy. Accidental injury of small bowel occurred in one case during minilaparotomy, which was treated by primary closure, followed by an uneventful postoperative period. In this series there was one case with severe hyperstimulation syndrome, which responded very well to conservative treatment.

Discussion

The rationale for GIFT is that it allows gametes, i.e. sperm and oocytes, to be placed directly into the natural physiologic environment appropriate for fertilization. The introduction of spermatozoa within the ampulla of the tube may overcome compromised sperm transport in patients with male or cervical factor infertility[10]. Patients with unexplained infertility and endometriosis may have some impairment in the oocyte pick-up mechanism or in the transport of gametes to the normal site of fertilization or may have coexisting luteinized unruptured follicle syndrome[11-13], all of which could be bypassed by the GIFT procedure[6,10].

In our study, twice as many ampoules of hMG were needed in the long versus the short protocol, probably reflecting the absence of endogenous pituitary contribution when hMG stimulation is begun after down regulation has been accomplished[9]. However, it is still debatable whether one protocol is better than the other. Our data seems to support Mettler et al[14] who reported a higher pregnancy rate using the long protocol started in the mid-luteal phase compared with the short stimulation. On the other hand, Zorn et al[15] and Frydman et al[16] could not demonstrate any advantage of the long versus the short protocol regarding folliculogenesis, oocytes recovered and pregnancy rates. Until the advantage of either protocol is established, we feel that the choice should be tailored according to the patient's convenience, cost, and side-effects.

To date, no reliable biochemical testing has been developed which can accurately and rapidly provide direct correlation to oocyte matura-

tional status[17]. Only morphological criteria are available for grading of oocyte maturity into 4 or 5 aspects, based upon the degree of dispersion of the cumulus/corona cells and the presence of a polar body[18]. However, classification of the oocytes under the dissecting microscope is difficult, and to a large degree imprecise and subjective[17,18]. Therefore, in our laboratory we simply characterize the oocytes as mature or immature (types I-II vs III-IV)[19], and find the method to be simple and satisfactory.

Because of concern about multiple pregnancies of higher order (≥ triplets), many fertility centres restrict the number of oocytes transferred to 4 or 5[6,19,20]. However, in a large series of 1071 women, Craft et al[21] demonstrated a continuous increase in the overall pregnancy rates with the number of oocytes transferred i.e. 13.9% for 1-2 oocytes, 24.7% for 3-4 oocytes, 38.1% for 5-6 oocytes, 42.8% for 7-8 oocytes, 38% for 9-10 oocytes and 50% when more than 10 oocytes were transferred. In their series, multiple pregnancies were also increased, but to a far lower extent than expected, particularly in those patients who yielded more than 10 oocytes, and the number of oocytes replaced did not affect the abortion or the ectopic pregnancy rates. Given that the ideal number of oocytes that should be transferred is not known, we decided to replace all mature oocytes to avoid the problem of surplus eggs. On the average, 4.7 oocytes were replaced, resulting in an overall pregnancy rate of 31.7% which is comparable to that of Craft et al[21] and others[6,18-20]. So far, there are two sets of twins out of the six clinical pregnancies, with no ectopic pregnancy. However, we agree that in places where cryofacilities are available, it may be advisable to replace only 4 oocytes for each GIFT cycle, with consideration given to cryopreservation of excess eggs for transfer in subsequent cycles. The question of whether gametes should be transferred to only one or both fallopian tubes is still unsettled. Although we observed more pregnancies when oocytes were replaced into both tubes, the numbers are too small to allow any definite conclusion. Evidence to date suggests that unilateral GIFT may be at least as successful as the bilateral approach, while the operating time is shorter and there is less risk of trauma to the fallopian tubes at the time of gamete transfer[22].

Most GIFT/IVF centres use hCG or progesterone for luteal phase support in cycles stimulated with combined GnRH-a and hMG to counteract the luteolytic action of GnRH-a[9]. In our program we prefer to use hCG because there is one recent prospective double blind randomized trial demonstrating the advantage of hCG over progesterone[23]. However, in the case of ovarian hyperstimulation syndrome, progesterone is preferable in order to avoid further stimulation to the muliple corpora lutea[9].

Conclusion

Although the number of patients in this report is small, it confirms the notion that GIFT can be successfully applied to couples with a history of long-standing infertility, who have failed other conventional treatments. Compared with IVF, GIFT is less sophisticated and more physiologic in that it mimics the normal events involved in fertilization. We feel that GIFT is an attractive alternative to IVF in cases in which the female partners have at least one patent fallopian tube.

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Gestational Choriocarcinoma with Brain Metastases : Treatment Results and Review of Literatures

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Abstract: *Fourteen patients diagnosed as gestational choriocarcinoma with brain metastases were reviewed concerning treatment results and prognostic factors. Most patients received combination chemotherapy concomitant with whole-brain irradiation. Six of fourteen patients (42.8%) survived and were well without evidence of disease from 9 months to 18 years after the diagnosis of complete remission. Poor prognostic factors include time interval from antecedent pregnancy to start of treatment more than 1 year ($p < 0.05$), modified WHO score more than 15 ($p < 0.05$), and craniotomy performed or not ($p < 0.05$). Furthermore, survivors are also affected by neurological manifestations, failed prior chemotherapy and site of brain metastases but it is not of statistical significance. Management strategies of gestational choriocarcinoma patients at risk of developing brain metastases include initial administration of intensive combination chemotherapy in all high-risk patients and those with lung metastases to eradicate all the tumours. For those who have brain metastases, multimodality approaches including whole-brain irradiation, intensive combination chemotherapy containing high dose methotrexate, and surgical removal in selected cases are recommended. Maintenance chemotherapy of at least 3 courses should be given after achieving remission to prevent recurrence. (Thai J Obstet Gynaecoly 1991;3:103-114.)*

Key words: gestational choriocarcinoma, brain metastases

Currently, gestational choriocarcinoma has been one of the most curable malignancies with the overall cure rate approaching 90% or more[1-5]. This is primarily related to the intrinsic sensitivity of this tumour to certain chemotherapeutic agents, the effective use of sensitive human chorionic gonadotropin (hCG) assays, the identification of high-risk factors that allow for individualization of therapy, and the aggressive use of multiagent che-

motherapy, radiation therapy, and surgery[6]. However, some groups of high-risk metastatic patients still have an unfavorable outcome, 10% to 25% of them would die from this disease[7-9], in 58% of which brain metastases is responsible[10]. Despite the intensive use of multiagent chemotherapy combined with whole-brain radiotherapy and surgery in selected cases, the prognosis is still poor. The survival is no more than 50%, especially in patients who develop brain metastases during treatment or relapse after an initial complete or partial remission[11-14].

The purpose of this report is to analyze some prognostic factors and survival of 14 patients with brain metastases of choriocarcinoma who were treated at the University of Texas MD Anderson Cancer Center between February 1968 and July 1990. On the basis of results and review of the literatures, the management strategies to maximize the chance of patient survival are proposed.

Materials and Methods

A medical record search was conducted to identify all patients diagnosed as choriocarcinoma with brain metastases. Some clinical profiles were analyzed and WHO scores were subsequently assigned as described in the previous report. Prior to 1972, patients were initially treated with single agent chemotherapy either methotrexate or actinomycin-D. Since 1972, patients were treated with a five-day course of methotrexate 25 mg intravenous daily, actinomycin-D 0.5 mg intravenous daily, and cyclophosphamide 5 mg/kg to a maximum of 250 mg intravenous daily (MAC). After 1976, folinic acid was added with increased dosage of methotrexate. Courses were administered after a seven to nine days interval or as soon as recovery from toxicity permitted. Chemotherapy was changed if hCG levels reached a plateau or began to rise on two successive values. Numerous single agents or combinations, including intra-arterial actinomycin-D, intrathecal methotrexate, modified Bagshawe regimen, combination of vinblastine, bleomycin, and cis-platinum (VBP), combination of etoposide, methotrexate, actinomycin-D, cyclophosphamide and vincristine (EMA-CO) and combination of bleomycin, etoposide and cis-platinum (BEP) were used in patients resistant to their first or second line treatment.

Most patients with brain metastases received 1500 to 3000 rads of megavoltage whole-brain radiotherapy over 10 days to 2 weeks (10 fractions), beginning simultaneously with the start of chemotherapy. Additionally, some patients were treated with radiotherapy for pulmonary, pelvic or hepatic metastases. Craniotomy, thoracotomy, hysterectomy and resection of their metastases were used in selected cases as indicated.

Response to therapy was monitored initially by weekly urine or serum bioassay for hCG. Since 1979, a radioimmunoassay for the beta subunit of hCG has been used. Patients were

considered to be in complete remission only after three normal weekly hCG titers. Patients who subsequently developed elevated titers were considered to have relapse. After three normal weekly titers, patients were followed with monthly titers for a period of one year; thereafter, six monthly measurement were made at least every 5 years or until the time of death. Additional follow-up included physical examination, chest x-ray, brain radionuclide or CT scan, and other indicated studies at various intervals.

Statistical analyses between groups were carried out by using log rank test and Wilcoxon rank test.

Results

All patients' treatment and outcome are summarized in Table 1.

Chemotherapy

All patients received systemic chemotherapy. One patient (LFT) received 2 courses of actinomycin-D combined with whole - brain radiotherapy and craniotomy, she survived with no evidence of disease and had 2 children later. After 1972, all patients were initially treated with multiagent chemotherapy, which was mostly the MAC regimen. Patients with progressive or drug-resistant tumours were individually considered for other regimens such as, VBP, modified Bagshawe regimen, EMA-CO, BEP and other experimental drugs. Intra-arterial actinomycin-D and cisplatin were used in two patients. Only two patients (TKT & RUN) received intrathecal methotrexate. One patient (VAP) was

Table 1 Summary of treatments and outcomes

Patients	Chemotherapy No. of courses	WBRT# (rads)	Craniotomy	Prognosis
LFT	2	2085	Yes	NED 18 yr.
CTM	7	3000	No	NED 2 yr. 4 mo.
KAD	22	2000	No	DOD 8 mo.
VAP*	60	2000	No	DOD 5 yr. 3 mo.
MJH*	14	2000	No	DOD 1 yr.
FR	20	5000	No	DOD 1 yr.
GD*	18	2000	No	DOD 1 yr. 1 mo.
PMG	21	4000	No	DOD 1 yr. 9 mo.
TKT	39	4700	No	DOD 2 yr. 1 mo
RUN	14	1200	No	NED 8 yr. 7 mo.
RDJ*	19	3000	Yes	DOD 2 yr. 1 mo.
MLS	7	3000	Yes	NED 10 yr.
KPC	17	-	Yes	NED 2 yr. 5 mo.
SKS	21	2880	Yes	NED 9 mo.

* = Developed brain metastases during treatment
\# = Whole-brain radiotherapy
DOD = Died of disease, NED = No evidence of desease

treated by immunotherapy with Bacillus Calmette Guerin (BCG) and husband's white blood cells, which resulted in a 3 fold rise of her serum hCG titer. Four patients received further maintenance chemotherapy after achieving first negative hCG titer. Recently, all patients would be treated with regimen containing higher dosage of methotrexate, in which the dosage was increased to 3.1 g/m^2 of body surface area, dividing 100 mg/m^2 given initially intravenously in 15 minutes and then followed by 3 g/m^2 intravenous infusion over 12 hours. Twenty-five milligrams of folinic acid, given every 6 hours, was also given 24 hours later. The side effects from this regimen were generally acceptable.

Radiotherapy

All but one patient (KPC) received whole - brain radiotherapy concomitant with chemotherapy as initial treatment in doses ranging from 1200 rads to 5000 rads. Additional courses of whole - brain radiotherapy were administered in 3 patients whose tumours persisted or progressed, one patient succumbed to intracranial bleeding. The other two died of extensive liver and lung involvement. Two (PMG & RUN) of four patients with liver metastases also received hepatic radiotherapy at doses of 2000 rads and 800 rads respectively, one died of massive intraabdominal hemorrhage from rupture of metastatic nodules. Furthermore, radiotherapy was also instituted to treat tumours in the lungs in 3 patients and the pelvis in 1 patient.

Surgery

During treatment, eleven of fourteen patients underwent at least one major operation. Craniotomy was performed in four patients for ventricular drainage (1)and tumour resection(3), all of them achieved complete remission. One patient's hCG (KPC) spontaneously returned to normal after undergoing tumour removal. Another patient (RDJ) who underwent suboccipital craniotomy, tumour resection could not be performed. She succumbed to brain stem herniation. Thoracotomy was performed on six patients for the purpose of removal of the resistant lung nodules in five patients, in another one, the tumours were unresectable due to extensive lung involvement and only lung biopsy was performed. Five patients underwent hysterectomy, but choriocarcinoma was identified in only one patient. D&C was performed in two patients who presented with vaginal bleeding, the results subsequently showed that the tumour still persisted in the uterus. In another patient (RUN) who received D&C, no tumour was identified but she came in with lower gastrointestinal bleeding and colonoscopy confirmed the metastatic nodules in the descending colon. One patient (MLS) underwent emergency laparotomy for small bowel perforations. The resected specimens revealed

metastatic choriocarcinoma in proximal jejunum and distal ileum.

Survival of the patients

Six of fourteen patients (42.8%) were alive and well without evidence of disease for periods ranging from 9 months to 18 years (mean 3 years) after the diagnosis of complete remission to the last follow-up. Among the surviving patients the longest duration of treatment was 18 months, utilizing a total of 17 courses of various chemotherapeutic regimens, including three major surgical procedures, and interestingly, without any radiotherapy (KPC). The shortest duration was 20 days, treatment included only 2 courses of actinomycin-D, craniotomy, and whole - brain radiotherapy (LFT). Among the dead group, the longest duration of treatment was 63 months, using a total 60 courses of various chemotherapeutic regimens, including immunotherapy, whole - brain radiotherapy, and two major operations but, without craniotomy (VAP). The shortest duration was 8 months, treatment administered including 24 courses of chemotherapy, two radiotherapies, and one major operation (KAD).

When survival by various WHO prognostic factors is separately analyzed (Table 2) to identify whether another factor had independently influenced the patients' survival, it is apparently seen that the group of patients who failed prior chemotherapy has a worse prognostic outcome. Sur-

Table 2 Survival by prognostic factors

Prognostic factors	No. of patients	Survivors		
		Number	%	
Age (years)				
≤ 39	13	6	46	
> 39	1	0	0	
Antecedent pregnancy				
Term	9	4	44	
Abortion	3	1	33	
Mole	2	1	50	
Interval				
≤ 1 year	7	5	71	$p < 0.05$
> 1 year	7	1	14	
Serum hCG (mIU/ml)*				
≤ 100000	5	1	20	
> 100000	7	3	43	
Prior chemotherapy				
Yes	5	0	0	
No	9	6	67	

*Two patients (LFT & CTM) were not evaluated

prisingly, only 1 of 5 patients whose serum hCG was 100000 mIU/ml or less survived, while 3 of 7 patients whose hCG titer was more than 100000 mIU/ml is still alive. However, two patients who are still alive are not included for evaluation. Type of antecedent pregnancy does not singificantly affect survival. The number of survivors in the term delivery group (44%) is nearly the same as those in combination of the other two groups (40%). Only one of seven patients attained remission when the interval was more than 1 year, compared with five of seven patients whose interval was less than 1 year survived. This difference is of statistical significance ($p < 0.05$).

Patients presenting with neurological signs or symptoms had a better prognosis, the survival rate was 57% compared with 29% in the non-neurological presenting group. With further analysis, it was discovered that four of the dead in the non-neurologic presentation group fell into the prior chemotherapy group and also developed brain metastases later. All four patients diagnosed with brain metastases during or after treatment died of the disease. Possibly, these may be the consequence of drug-resistant tumours, delay in diagnosis and treatment. Interestingly, three of these four patients had been initially treated with MAC prior to diagnosis of brain metastasis. Furthermore, three of the six survivors in the brain metastases presentation group had failed MAC chemotherapy before going into complete remission with other chemotherapeutic regimens. Only one survivor was successfully treated with MAC.

With a limited number of patients, it was found that if the size and number of brain metastases are not considered, the patients who present with brain stem seeding tend to have a worse prognosis. This is, probably, because it contains various vital centers that control circulatory and respiratory functions, even a small lesion can cause fatality. Furthermore, operation in this area is difficult and dangerous to be performed.

Further analysis was also carried out according to the modified WHO prognostic score. All those high-risk patients are, subsequently, subclassified into two groups, those with scores of 15 or less and those with scores of more than 15. The survival rate was 63% in the former and 17% in the latter groups ($p < 0.05$).

When considering survival after craniotomy was performed, it is clearly seen that the surgery group has a better prognostic outcome. The survival rate in the surgery and non-surgery groups was 80% and 22% respectively ($p < 0.05$). All five patients in the non-craniotomy group with modified WHO score of more than 15 died of the disease and also had uncontrollable brain lesion. Among the seven patients who died of diseases in the non-craniotomy group, two died from direct intracranial hemorrhage, three remained in uncontrolled brain tumours, but died from other tumour - related causes (liver and respiratory

Table 3 Causes of death

Patients	Final report
KAD	Increased hCG titer, uncontrolled brain lesion, liver failure due to extensive tumour metastases.
VAP	Progressive brain lesion, intracranial hemorrhage.
MJH	Increased hCG titer, uncontrolled brain lesion, expired at home.
JFR	Increased hCG titer, uncontrolled brain lesion, intracranial hemorrhage.
GD	Increased hCG titer, uncontrolled brain lesion, respiratory failure due to extensive lung involvement.
PMG*	Massive intraabdominal hemorrhage from rupture of metastatic tumour of the liver, diffuse metastatic tumour replacing approximately 90% of the liver; extensive lung involvement; massive infiltration of the splenic sinusoids.
TKT	Increased hCG titer, respiratory failure due to extensive and uncontrolled lung lesions.
RDJ#	Progressive brain lesions, cardiopulmonary arrest due to herniation of brain stem; choriocarcinoma in medulla oblongata.

* Autopsy performed but permission for CNS examination was not granted
# Autopsy performed

failure) and the other two died of extensive lung and liver involvement. The only one patient who died in the craniotomy group had a lesion in the brain stem.

Table 3 lists the causes of death in eight patients. Six still had uncontrolled brain lesions documented by clinical features, elevated hCG titer, radiologic examinations and autopsy finding. Two patients died of extensive liver involvement and two died of massive lung involvement.

Discussion

Thus far, no consensus concerning proper and effective therapeutic regimens has been established for management of choriocarcinoma patients with brain metastases. In this institute, various treatment modalities have been employed according to patients' conditions, medical advancement and physician discretion.

Seven of fourteen (50%) patients presented with primary neurological signs or symptoms. Four of these patients of whom three had undergone craniotomy are alive without evidence of disease. It is probably because early diagnosis and proper treatment can be instituted in these patients when compared to the non-neurologic presentation group. Nine of fourteen (64%) patients diagnosed as choriocarcinoma with brain metastases occurred after term delivery. Antecedent term delivery is determined by some authors as an independent poor prognostic factor due to a prolonged preclinical period for tumour growth[15]. These patients, from this study, tended to have a longer interval when compared with the other types of pregnancy, but the survival does not sig-

nificantly differ. The results confirm the findings of Jones et al[12]. Interval between the end of antecedent pregnancy and the start of treatment has significant impact on the survival in this study. Those with an interval of 1 year or less had a better prognostic outcome than those with more than 1 year ($p < 0.05$). Level of serum hCG which reflects tumour burden does not affect survival among this group.

In patients whose modified WHO score is 15 or less, only one in eight (12.5%) could achieve complete remission with MAC chemotherapy. In addition, three of four patients developed brain metastases while on MAC regimen and three of the six survivors had failed this regimen before attaining complete remission with other salvage regimens. It seems questionable whether MAC regimen is virtually effective in cases of brain metastases. A report from Memorial Hospital[12] revealed that 12 of 19 patients had received MAC regimen prior to diagnosis of cerebral metastases. In contrast, a report from Charing Cross Hospital[11], utilizing EMA-CO regimen to treat patients with cerebral metastases showed satisfactory results with 72% survival rate. None of the study group with a score of more than 15 was cured by combined chemotherapy and whole - brain radiotherapy. Only one patient survived with additional tumour resection. Consequently, early neurosurgical intervention in patients with a score more than 15 is recommended if the lesions are accessible for removal without undue risks.

Since successful treatment in patients with brain metastases by 2000 rads whole - brain radiotherapy (WBRT) in addition to chemotherapy was reported at the National Cancer Institute in 1968[16], most centers have adopted this modality in their treatment schemes[10,12,17-19]. In a collected series of 228 patients with cerebral metastases, Jones[20] found that the survival in patients receiving WBRT was 37% compared with 16% for non-irradiated patients. Since choriocarcinoma is known to be both radio- and chemosensitive, intensive combined modality is definitely justified. Whether WBRT acts alone or synergistically with chemotherapy is not well documented. The only evidence that WBRT and not chemotherapy alone plays a major role in eradicating cerebral lesions derived from the autopsy findings that no tumour was identified in the brain while a tumour in other metastatic organs still persisted[16,21]. Not only does WBRT have a tumouricidal effect but also exerts a hemostatic effect on choriocarcinoma which can reduce the risk of intracerebral hemorrhage. This can allow the patients to receive further intensive chemotherapy. Due to the high incidence of multiple metastases, radiation fields usually encompass the entire intracranial contents 3000 rads WBRT over 10 fractions is recomended to be administered without delay in conjunction with simultaneous multiagent chemotherapy[10,17-18]. Since radiation only kills cells as they attempt mitosis, and normal neurons

are a nondividing tissue in adult life and therefore do not express radiation damage. Alopecia is an inevitable consequence but hair will regrow if the patient survives long enough[22]. No gross intellectual impairment was found in the series of Weed and coworkers[13].

Recently, higher dose of methotrexate (3.1 g/m^2) given by 12 hour intravenous infusion, followed subsequently by folinic acid was used to treat these patients in this center. Satisfactory results were encountered despite not having intrathecal methotrexate. Reports from Charing Cross Hospital[11] advocated intrathecal methotrexate as part of an initial combination chemotherapy with the reasons that higher drug concentration will be achieved in the CSF than by any other means, and, also the tumour will receive at least one drug both from the systemic side and from the CSF in potentially high concentration[11,14]. However, high dose systemic chemotherapy could achieve the same results[1,13]. Following conventional intravenous administration of methotrexate, the compound minimally enters the brain, with a CSF: plasma concentration ratio of 0.0006, but with high dose 24 hour methotrexate infusion the ratio has been shown to increase up to 0.03 (50 times) reaching the cytocidal concentration levels[23]. A water soluble drug could exchange readily between the blood and tumour tissue as long as a constant drug concentration was maintained in plasma, lending support to the use of prolonged intravenous infusion chemotherapy rather than bolus therapy and negating the need for intrathecal methotrexate in patients with brain metastases. No available data indicate that intrathecal methotrexate penetrates satisfactorily into tumours deep in the brain. Furthermore, ventricular methotrexate concentration administered by intralumbar route, varied considerably from patient to patient despite similar doses[24].

Despite the multimodality approach, the overall survival rate in this study is only 43%. None of the four patients who developed brain metastases during treatment survived. This may imply that the tumour is already drug-resistant. Table 4 shows the results of treatment in other series. It is clearly seen that patients who develop brain metastases during treatment or relapse after complete or partial remission have a grave prognosis. Rustin et al[14] reported 29% survival rate in this patient group with additional surgical removal of tumour combined with EMA-CO regimen. Bagshawe[25] advocated that surgical removal will offer the best chance of eradicating cerebral metastases which occur during the course of chemotherapy.

Of five patients who had undergone neurosurgical procedures, complete tumour resection could be accomplished in three. All are alive without evidence of disease. One patient (LFT) having a lesion at the brain stem received only ventricular drainage. She is still alive. Surgical removal could not be performed in

Table 4 Survival by mode of brain metastases presentation

Authors	Prior to treatment		During treatment/Relapse	
	No. Patients	Survivors (%)	No. Patients	Survivors (%)
Lurain et al[1]	16	8 (50)	13	0 (0)
Weed et al[13]	11	7 (64)	12	3 (25)
Ishizuka et al[26]	7	1 (14)	20	0 (0)
Athanassiou et al[11]	33	16 (49)	36	2 (6)
Rustin et al[14]	18	13 (72)	7	2 (29)
Jones et al[12]	4	1 (25)	15	4 (27)
Present study	10	6 (60)	4	0 (0)
Total	99	52 (52)	107	11 (10)

another patient who also had brain stem metastases. She succumbed to brain stem herniation later. Ishizuka et al[26] divided brain metastases into 4 categories; superficial, intermediate, deep and cerebellar. Deep type (basal ganglia, brain stem, etc.) occcurs in only 10% and appears to be inaccessible to surgical removal.

As generally accepted, emergency craniotomy is recommended in patients who present with acute deterioration of intracerebral hemorrhage[18,26,27]. Some authors advocated early surgical removal if the lesion appears solitary, certainly localized, and resectable without causing neurological deficit[14,28]. Since 60-80% of metastatic brain tumours are solitary and localized in the superficial layer of the cerebral cortex[10,26,28], the lesions are, possibly, accessible to successful surgical removal. Chemotherapy should also be given in conjunction with surgery to prevent further dissemination of tumour cells during manipulation. Surgical removal is also recommended in cases of drug-resistant tumours[14,26]. Early neurosurgical intervention should be considered in these very high-risk patients who fulfill the criteria for surgical removal.

In conclusion, management strategies of GTT patients at risk of developing brain metastases and those in whom brain metastases have already been diagnosed are proposed as follows. From the preventive aspect, intensive systemic chemotherapy should be initially instituted in all high-risk patients to eradicate all the tumours and minimize the chance of developing drug-resistant tumour. Regimens containing high dose methotrexate are recommended. Since brain metastases are always secondary to lung metastases, consequently, all patients with lung involvement should be administered intensive systemic chemotherapy from the start of treatment.

Should evidence of drug-resistance appear, a salvage regimen is promptly instituted. Surgical excision may be indicated in selected cases.

From the therapeutic aspect, a multimodality approach composed of WBRT in conjunction with intensive systemic chemotherapy is the treatment of choice. Early craniotomy may be considered in cases of a solitary lesion which appears resectable without causing severe neurological deficit to the patients. All patients with a history of failed prior chemotherapy or evidence of drug-resistant tumour, surgical tumour resection is recommended, especially in those whose metastatic tumours elsewhere have disappeared. Neurosurgical intervention is certainly the treatment of choice for patients developing rapid neurologic deterioration. After achieving negative hCG level, patients should receive maintenance chemotherapy at least 3 courses to prevent recurrence. Closed follow-up with sensitive hCG measurement must be employed every 1-2 weeks in the first three months, then monthly for one year and six monthly thereafter to confirm complete remission and early detection of relapse or late recurrence.

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Dynamic Computerized Tomography of Pelvic Masses

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Abstract : *The performance of consecutive, 4.8 sec computed tomographic (CT) scans, after the intravenous administration of a bolus of amidotrizoate, was found to be useful in evaluating the vascular anatomy and organ perfusion in benign and malignant pelvic masses. It appears to have significant advantages over conventional intravenous contrast enhancement CT scan in selected cases. (Thai J Obstet Gynaecol 1991;3:115-119.)*

Key words : computerized tomography, pelvic mass

X-ray computed tomography (CT) is an easy, safe and valuable diagnostic method for tumours in the pelvic cavity because it is a relatively noninvasive procedure[1,2]. However, the use of a low concentration drip infusion of iodinated intravenous contrast medium in routine CT, which is delayed for several minutes after intravenous contrast medium administration, may obscure subtle areas of abnormality in the CT of the body[1]. Dynamic CT, which is performed by obtaining rapid sequential CT scans following a bolus injection of intravascular contrast medium, has been shown to be useful in the analysis of pathological lesions, such as cerebral[3], hepatic, biliary or renal lesions[4,5]. However, dynamic CT has seldom been utilized in gynaecologic diseases. We have evaluated patients with benign and malignant pelvic masses with dynamic CT, and found it to have some advantages.

Materials and Methods

A GE/Yokogawa 9000 CT/T scanner was utilized in performing the dynamic scanning series. In patients who were evaluated by dynamic CT scanning, the level for the dynamic CT was selected from conventional CT scans obtained through the general area of anatomic interest prior to the administration of intravascular contrast medium. After selection of the appropriate level, a dynamic CT scan was performed.

An 18-gauge Teflon needle was inserted into an antecubital vein, and 40 ml of contrast material (60% meglumine and sodium amidotrizoate) was injected at a flow rate of 8 ml per second. The scanning was begun 8

sec after the initiation of the bolus injection; i.e. a sequence of 6 exposures, each 4.8 sec in duration, with an interscan interval of 1.5 sec, followed by 3 exposures, each 4.8 sec in duration, interscan interval of 15 sec.

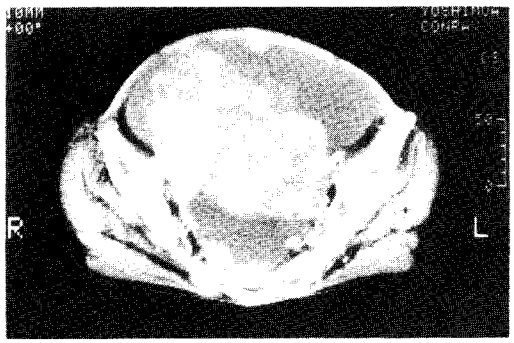
Analysis of the dynamic CT scans obtained during the intravascular passage of the contrast bolus provides a nearly real-time analysis of the pharmacokinetics of the contrast medium as it passes through the tissue included within the scan slice. For patients evaluated with this modality illustrate the potential value of this methodology.

Cases Report

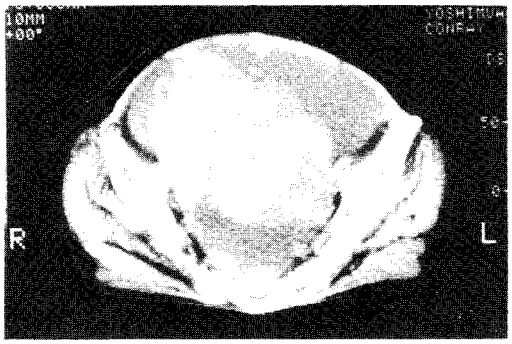
Case 1

A 75-year-old woman had a primary ovarian tumour (serous cystadenocarcinoma, Stage T3NxMO) that had been partially resected 2 years earlier. The patient was given 4 courses of double chemotherapy with cis-diamminedichloroplatinum (CDDP) and adriamycin. She had recently developed symptoms of abdominal distension, and her serum CA 125 level was elevated (14500 U/ml). A CT scan was done to evaluate the possibility of a recurrent ovarian cancer. Dynamic CT revealed ascites and a mass in the lower abdomen which was highly vascularized and had prominent solid and cystic components, which were highly suggestive of recurrent ovarian cancer (Fig. 1).

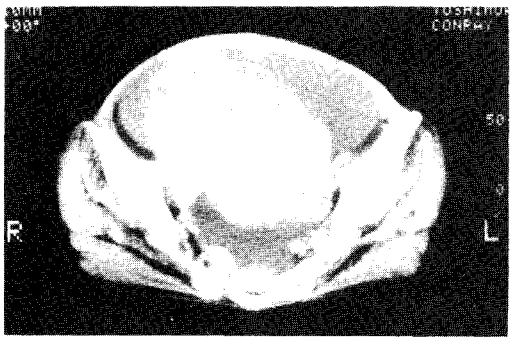
Fig. 1 Case 1. CT image in 75-year-old woman with suspected recurrent ovarian cancer.



(A) Prior to the intravenous injection of the contrast material.



(B) Dynamic 4.8 sec CT scan initiated 21 sec after the beginning of an intravenous bolus injection of contrast material, showing a late arterial phase. The tumour shown in this picture is highly vascularized and has many necrotic areas.



(C) Dynamic 4.8 sec CT scan initiated 73 sec after the beginning of an intravenous bolus injection of contrast material, which is identical with the CT image obtained after conventional drip infusion of iodinated intravenous contrast medium.

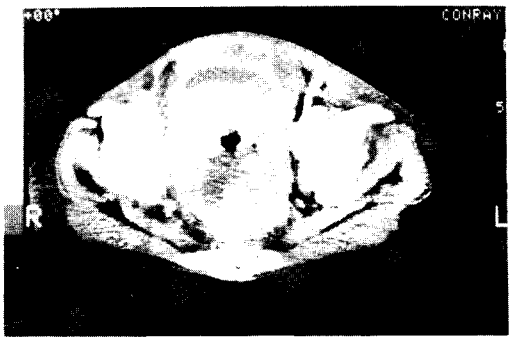
Case 2

A 60-year-old woman had a primary ovarian tumour (serous cystadenocarcinoma, Stage T3NxMO) that had been resected 3 years earlier, and she had been given 4 courses of double chemotherapy with CDDP and adriamycin. She had recently developed symptoms of abdominal distension and an elevated serum CA 125 level (1200 U/ml). Her left kidney had already been autonephrectomized and a right ureteral obstruction, which had been demonstrated by drip infusion pyelography, required nephrostomy. There were no pathological findings in the mucous membrane of the urinary bladder cystoscopically. A CT scan revealed a large necrotic mass, which was clearly distinguished from the surrounding ascites by contrast enhancement with dynamic CT scan. Abnormally high vascularity extending into the right vesical wall was demonstrated. These findings strongly suggested recurrence of her ovarian cancer (Fig. 2).

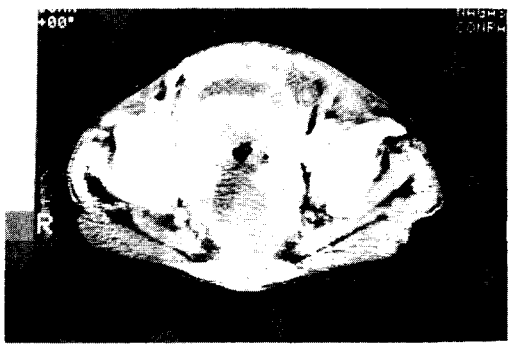
Case 3

A CT scan was obtained in a 45-year-old woman with a 3 - year history of a tumour in the lower abdomen. She was in the 5th week of gestation. Since her pregnancy was determined to be terminated by social indication, dynamic CT was performed and an enlarged uterine body with a gestational sac was revealed. Opacification of the left uterine artery was shown, and homogeneous tumour arising from the uterine body was enhanced immediately thereafter. No necrotic lesions or disequilibrium in contrast material distribution were observed. The postoperative histologic diagnosis was uterine leiomyoma and decidual change of the endometrium (Fig. 3).

Fig. 2 Case 2. CT image in 60-year-old woman with suspected recurrent ovarian cancer.



(A) Dynamic 4.8 sec CT scan initiated 8 sec after the beginning of an intravenous bolus injection of the contrast material. Note both femoral arteries are opacified.



(B) Dynamic 4.8 sec CT scan initiated 27 sec after the beginning of an intravenous bolus injection of the contrast material shows a late arterial phase. Note the abnormal vasculature extending into the right vesical wall. The tumour image is clarified by the dynamic CT scan.

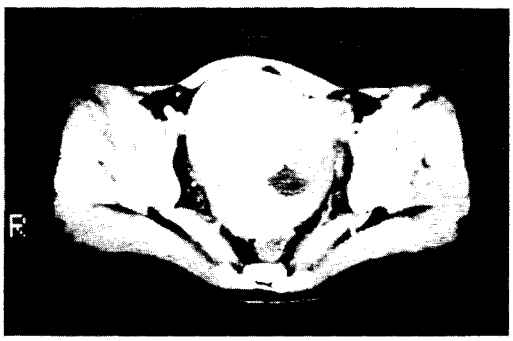


Fig. 3 Dynamic CT scan in 45-year-old woman with myoma uteri and 5th week of gestation. A continuous dynamic 5 sec CT scan initiated 14 sec after the beginning of an intravenous bolus injection of the contrast material is shown.

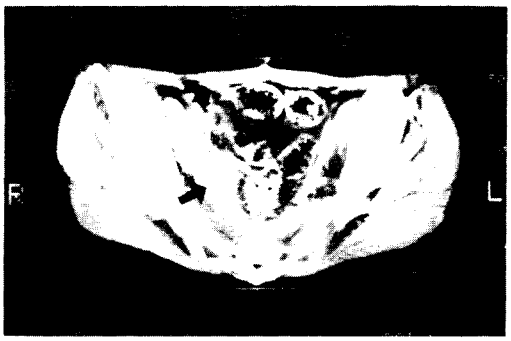


Fig.4 CT prior to the contrast enhancement features a tumour along the pelvic side wall, suggesting lymphadenopathy. The dynamic CT scan was not effective in contrast enhancement of these nodes.

Case 4

A 40-year-old woman had a primary adenocarcinoma of the uterine cervix (Stage T1bN0M0) that had been treated with radical hysterectomy and postoperative irradiation a year earlier. She had recently developed lumbago and ureteral obstruaction was demonstrated by intravenous pyelography. A CT scan was done to evaluate the possibility of a recurrent uterine cancer. Conventional CT scan revealed pelvic adenopathy, however, dynamic CT scan was not effective in contrast enhancement of this enlarged lymph-node (Fig. 4).

Discussion

The case examples presented indicate that valuable information regarding blood vessels, perfusion, and amidotrizoate pharmacokinetics can be obtained during dynamic CT. The advantage of this type of analysis is that it can be performed after intravenous contrast medium administration without the utilization of intraarterial catheters. It also appears to be useful in detecting mass lesions and in accurately defining the extent of disease. Bolus contrast medium administration still allows subsequent conventional postcontrast nondynamic CT. This technique is recommended for monitoring the response of gynaecologic malignancies to therapy. The recent introduction of nuclear magnetic resonance imaging seems to improve the imaging of the tumor in the abdomen[6], however, the cost of the equipment is prohibitive for its generalized use in patients with pelvic masses. Therefore,

rapid scanning of the X-ray CT after bolus contrast medium administration may be the best method for performing these types of studies.

There are limitations in the clinical applications of dynamic CT, including an increased dose of radiation to the patient and the potential complications of administering large amounts of high osmolality iodinated contrast media. Information from consecutive scans may only be obtained at a chosen level with present technology. Conventional CT has been reported to be useful in the diagnosis of pelvic lymphadenopathy[7], dynamic CT scan is not considered to have any additional advantage in the imaging of lymphadenopathy, which is not considered to be abundant in vasculature. However, dynamic CT in conjunction with bolus intravascular contrast medium administration appears to be an effective diagnostic imaging technique and should probably be employed more frequently in diagnostic CT imaging.

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A Case of Congenital Cystic Adenomatoid Malformation of the Lung (CCAM)

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Abstract : *A case of the fetal intrathoracic abnormality with ascites detected by ultrasonography at 27 weeks of gestation was demonstrated to be congenital cystic adenomatoid malformation of the lung after delivery. The prognosis of the infant after the resection of the tumour was favourable. This case was reported and the problems of diagnosis and management were discussed.(Thai J Obstet Gynaecol 1991;3:121-125.)*

Key words : cystic adenomatoid malformation, congenital anomaly, fetal ascites, amniocentesis

Congenital cystic adenomatoid malformation of the lung (CCAM) has been reported with increasing frequency with regard to prenatal diagnosis by ultrasonography in the past few years[1]. This malformation was classified histologically into three types by Stocker et al[2] and its spectrum of clinical presentations is broad by the extent of the affected pulmonary segment or lobe[3]. Our report describes the prenatal detection of fetal intrathoracic abnormality with ascites at 27 weeks of gestation, followed by the surgical excision, revealing a type II lesion of CCAM.

Case Report

A 31 years old woman (gravida 2, para1) was referred to our hospital at 27 weeks gestation because of fetal ascites detected by ultrasonography (Figure 1). A more detailed examination demonstrated maternal polyhydramnios, and a large, homogenous, solid mass occupying the fetal left hemithorax with mediastinal shift to the right (Figure 2). The fetal growth was appropriate for dates of gestation and the NST was reactive. Amniocentesis was performed. Alpha - fetoprotein (AFP) levels in the amniotic fluid

were not elevated at 2060 ng/ml and cytogenetic analysis showed a normal 46 XX karyotype. Fetal ascites were aspirated and the fluid was serous and no germs were detected by culture. Amniographic examination revealed no surface malformation of the fetus. These findings suggested that the diagnosis was likely to be CCAM in the left hemithorax, but herniation of the fetal diaphragm was not completely ruled out.

Preterm premature rupture of membranes occured at 30 weeks and 4 days of pregnancy and the volume of amniotic fluid was diminished, followed by variable deceleration of the fetal heart rate. Cesarean section was performed because of fetal distress. A 1552g female infant was delivered. The pH value in the umbilical artery was 7.29, and Apgar scores were 5 at 1 min and 9 at 5 min.

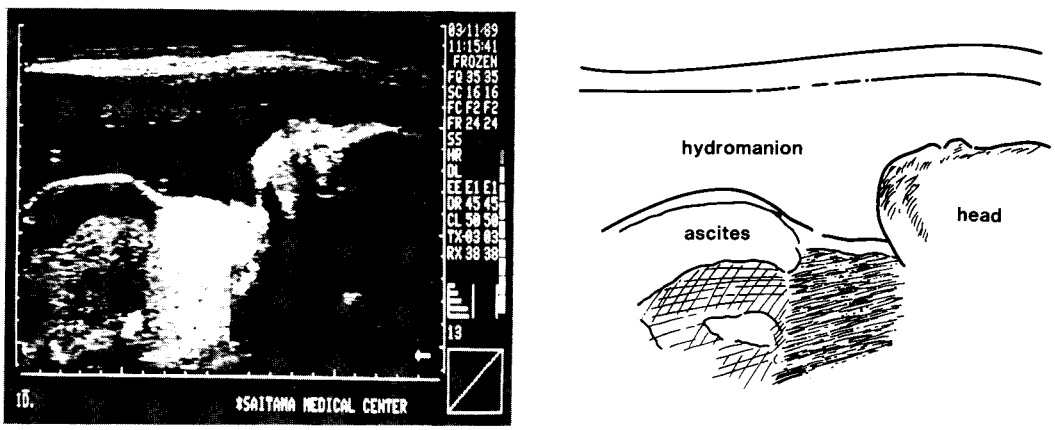

Fig. 1 Polyhydramnios and fetal ascites detected by ultrasonography.

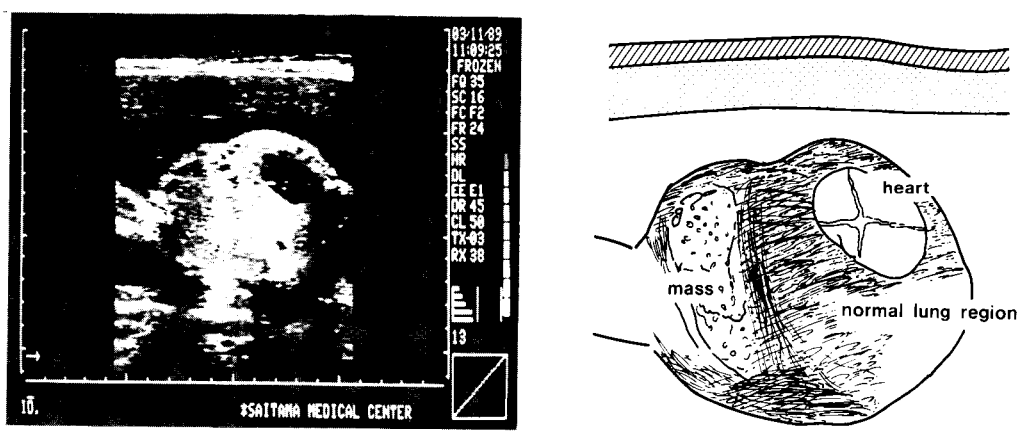

Fig. 2 The left thoracic mass, shifting the heart to the right side, detected by ultrasonography.

Immediate intubation and respiratory management were effective to overcome the respiratory distress for the first few days. Chest X-rays, ultrasonography, and CT scan revealed a mass in the left lower lobe, entirely consistent with a diagnosis of CCAM (Figure 3). A left lower lobe resection was performed on day 39 because of the development of respiratory distress caused by the compression of the lung by the mass. Postoperative course of the infant was good and on follow up visit, the infant was judged to be growing normally. Upon gross examination, the cut surface of the resected lobe showed numerous, small cysts filled with mucinous fluid (Figure 4). Histological examination of the mass revealed a lot of small cysts of varying size that were lined by ciliated columnar cells (Figure 5). Cartilage and inflammatory lesion were not present, whereas, some bands of smooth muscle were found. The pathologic diagnosis was CCAM, Stocker type II.

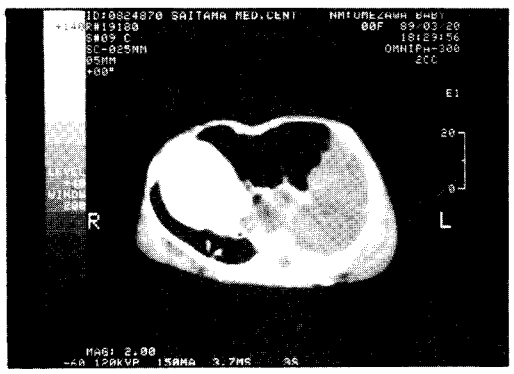

Fig. 3 Computed tomography taken on day 2 after birth demonstrates a marginal clear-cut mass in the left posterior part of the lower lobe consistent with a CCAM.

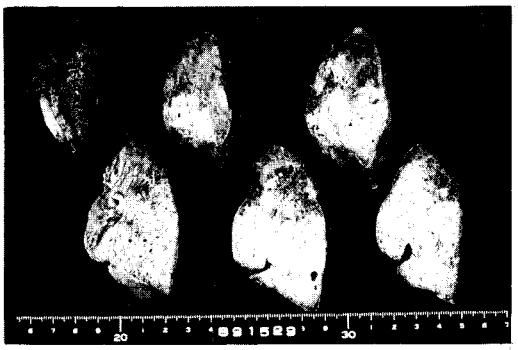

Fig. 4 Macroscopic findings of the resected lung mass show many microcysts with mucus, revealing type II CCAM.

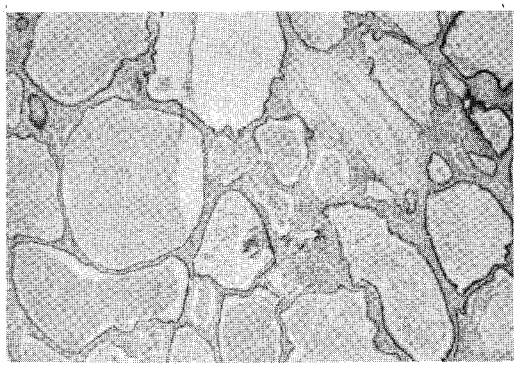

Fig. 5 Histopathological findings of the mass show small cysts lined by ciliated columnar cells looking like bronchal cell and scattered of residual lying tissue in the cyst (x100).

Discussion

Congenital adenomatoid malformation of the lung was first described by Ch'in and Tang in 1949[4]. Several reports on the rare congenital cystic disease of the lung discussed the nature of the malformation and the type of pathologic lesion[5,6]. In 1977, Stocker et al[2] categorized the lesion by clinical, gross, and microscopic

Table 1 Stocker's classification

Types		Prognosis	Frequency
I	single or multiple large cysts (>2cm in diameter)	: with no associated anomalies better prognosis than others	75%
II	multiple cysts (<1cm in diameter)	: frequently associated with other congenital anomalies	10-15%
III	bulky non-cystic lesion	: invariably poor with stillbirth or neonatal death	10%

findings into three types : cystic, intermediate and solid (Table 1). This case was classified as type II, of which the incidence was 10-15% of CCAM. Although the prognosis of type II, on the whole, is rather poorer because of the high frequency of associated congenital anomalies, this reported case showed as good a prognosis as seen in type I[3]. The correct diagnosis of CCAM in utero is of importance to the patient. The diseases taken into consideration as differential diagnosis are listed in Table 2[7,8]. Diaphragmatic hernia has to be excluded since it shows a similar clinical picture. Ultrasonography is a very useful tool in the diagnosis of CCAM, and MRI has recently been advocated as a very powerful and precise tool to detect CCAM. Furthermore, AFP levels in the amniotic fluid or in umbilical cord blood have been reported to be high in patients with CCAM[9,10] because of tumour-like lung masses of embryonic origin, though no elevation of AFP was shown in this case. In order to permit therapeutic planning, the early diagnosis by ultrasonography is of utmost importance. The presence of maternal hydramnios, in general, is a strong indication of poor prognosis as well. Therefore, its intensive prenatal care of the patient by the fetal heart monitoring, prevention of premature labour and the frequent of the polyhydramnios and fetal ascites, is mandatory to decide the mode of delivery and its timing of termination of pregnancy. After delivery, the prognosis of the newborn depends on the type and extent of its malformation as well as of maturity and hypoplasia of the unaffected lung. Therefore, avoidance of fetal distress has to be considered during vaginal delivery, and cesarean section is the choice, if fetal distress is expected. Surgical resection is the treatment of choice in the newborn

Table 2 Differential diagnosis

1. Mediastinal origin
   teratoma, enteric cyst, lymphangioma, meningocele
2. Congenital diaphragmatic hernia
3. Pulmonary origin (rare)
   CCAM, giant lobar emphysema, bronchial atresia
4. Cardiac origin (rare)
   rhabdomyomata

infant, when it has emergency indication[11]. In this case, the newborn baby was under observation in the NICU, in which the complication of sepsis and DIC took place after the delivery and the dyspnea progressing indicated lobectomy at 39 days of age. Complete recovery was accomplished by the procedure.

In conclusion, the proper diagnosis of CCAM in utero by ultrasound is of importance to permit the therapeutic planning after delivery with good survival rate.

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7th Scientific Annual Meeting

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SPECIAL ARTICLE

Viral Carcinogenesis of the Cervix

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Since the initial report in 1977 by Meisels et al[1] linking pre-invasive squamous lesions of the cervix with an infection by the Human Papillomavirus (HPV), there has been a rapid expansion of knowledge in the field of viral carcinogenesis of the cervix. Much of the increased knowledge was only made possible by concomitant developments in recombinant DNA technology.

The purpose of this review is to summarize current concepts of viral carcinogenesis of the cervix. Secondly, a major purpose is to present implications for cervical screeening and management based on these concepts.

Machanism of Viral Carcinogenesis

It is now widely accepted that an infection of the cervix by an oncogenic strain of HPV is necessary to cause oncogenic transformation[2-4]. More than 60 strains of HPV have now been identified but only about 20 affect the lower female genital tract. Of these 20 strains, only some are associated with cervical carcinoma, the common ones being HPV 16, 18, 31, 33 and 35. The common non-oncogenic strains are HPV 6 and 11. The fundamental difference between oncogenic and non-oncogenic HPV strains is the ability of the former to integrate part of its genome into the genome of the host epithelial reserve cell. That part integrated is constant for each of the oncogenic strains and includes the E6 and E7 genes along with their upstream regulatory region (URR) which controls their activity (i.e. transcription of viral DNA)[5,6].

Only very recently has it been discovered how this integration of the viral genes E6 and E7 effects malignant transformation. Within the human genome there are probably numerous "anti-oncogenes" whose role is to control normal cell proliferation and differentiation. Two such genes include the RB and p53 genes. (The RB or retinoblastoma gene was so named because it was previously found that a defect or mutation was associated with the development of retinoblastoma). These "anti-oncogenes" each produce a protein whose role is to control normal cell proliferation and differentiation. However, the activated E6 and E7 genes each produce a protein product which preciptates and inactivates

the protein product of the p53 and RB genes, respectively[7,8]. As a consequence, control over cell proliferation and differentiation is lost.

Thus, cervical carcinogenesis begins with the integration of viral DNA into the cervical epithelial reserve cell. From that point on, there is a progressive loss of control of cell proliferation and differentiation with each stepwise loss being inherited by the daughter cells. Ultimately, an autonomous clone of cells emerges with the capacity to invade and metastasize.

Pre-invasive Carcinoma

Before a clone of invasive cancer cells emerges, there is a continuum of morphologic changes which reflects the progressive loss of control of cell differentiation, or pre-invasive carcinoma. The older terminology of *Dysplasia/Carcinoma in situ* divided that continuum into four groups as shown in Figure 1A. In that terminology malignant transformation was believed to occur between severe dysplasia and cacinoma in situ.

The term "Cervical Intraepithelial Neoplasia" (CIN) was introduced by Richart[9] in 1967 to describe the same continuum of pre-invasive histologic and cytologic changes of the cervix associated with SCC. These changes were arbitrarily divided into three groups with CIN III including both severe dysplasia and carcinoma in situ (CIS) (Figure 1B). This terminology rapidly replaced the older dysplasia used in many centres. Its fundamental concept that all degrees of CIN are neoplastic has been subsequently validated by recent discoveries of viral oncogenesis : malignant trans-

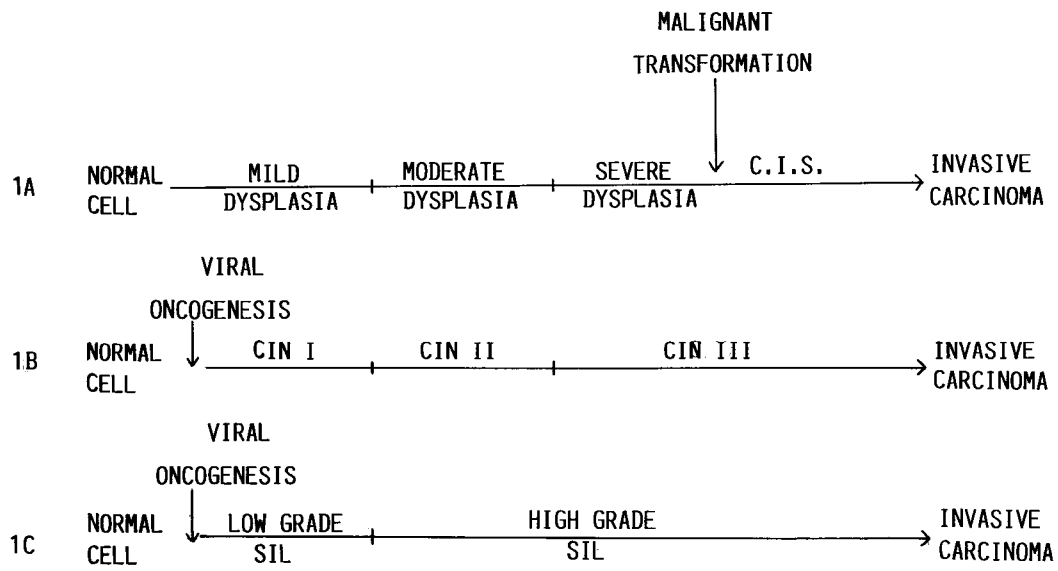

Fig.1 Terminology used to describe pre-invasive cervical SCC.

formation occurs with integration of viral DNA before any morphologic changes are apparent.

However, there is a high diagnostic error rate associated with CIN I. A variety of artefacts all produce slight nuclear changes which are indistinguishable from true CIN I on a Pap smear. These artefacts include infections (e.g. Candida, Trichomonas, various bacteria), chronic irritation (from the string of an intra-uterine contraceptive device), prior irradiation, etc.. Also, an infection by a non-oncogenic HPV strain commonly results in slight nuclear changes reported as CIN I. But, none of these false positive cases of CIN I has the capacity to progress to invasive disease.

In recognition of the high diagnostic error rate associated with CIN I, and in an attempt to further standardize the terminology, the "Bethesda System"[10] has recently introduced the term "Squamous Intraepithelial Lesion" (SIL). The same continuum of morphologic changes would be divided into only two categories: low grade SIL corresponding to CIN I, and high grade SIL corresponding to CIN II and III (Figure IC). (For a review of the controversies surrounding the Bethesda System, see reference 11).

Implications for Cervical Screening and Management

The basic principle of management for a woman whose Pap smear is reported to show any degree of CIN has been to refer that woman for colposcopy. However, the high diagnostic error rate associated with CIN I, the epidemic of cervical HPV infections, and the need to contain health care costs have all forced a re-assessment of that approach. The Third Canadian Task Force on Cervical Cancer Screening Programs is expected to soon recommend[12] that women whose Pap smears are reported as CIN I should have repeat smears every six months for up to 24 months. Only those women whose smears progress to CIN II or III, or those whose CIN I persists at the end of 24 months should be referred for colposcopy.

Realizing that there is a lag period of several years between viral integration (malignant transformation) and the development of invasive disease, the Task Force is also expected to recommend that after two normal satisfactory smears one year apart, women be re-screened only every third year. (For a review of the Task Force's recommendations, see reference 11). This is similar to recent screening recommendations suggested by the American Cancer Society[13].

The Future

Further advances in viral oncogenesis may reasonably be expected in the future to alter our approach to screening and management of cervical SCC. Will a vaccine some day be available to prevent this disease ? Advances in DNA technology, for example, may convert traditional cy-

tology screening into a biochemical test for the detection of integrated E6 or E7 or their protein products.

Further knowledge of the world-wide epidemiology of oncogenic HPV strains is also needed. Are there subtle differences in the behavior of carcinomas derived from various oncogenic HPV strains ? Until the answer to the latter question is clear, modifications to the above screening and management recommendations may be necessary based on local experience considering local resources and expertise[14].

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Authors Index

Subject Index