ISSN 0857-6084

THAI JOURNAL OF OBSTETRICS AND GYNAECOLOGY

THE OFFICIAL JOURNAL OF
THE ROYAL THAI COLLEGE OF OBSTETRICIANS
AND GYNAECOLOGISTS

VOL. 7 NO. 1 JANUARY - JUNE 1995

Thai Journal of Obstetrics and Gynaecology

ISSN 0857-6084. **The Official Journal of the Royal Thai College of Obstetricians and Gynaecologists.**

Vol. 7 No. 1 **January - June 1995**

CONTENTS :

Outcome of Pregnancies with First Trimester Threatened Abortion : A Prospective Study

Theera Tongsong MD,
Jatupol Srisomboon MD,
Thongchai Polsrisuthikul MD.

Division of Maternal Fetal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

Abstract : ***Objective** : To evaluate the outcome of pregnancies complicated with first trimester threatened abortion. **Study design** : Prospective study of 224 singleton pregnancies complicated with first trimester threatened abortion undergoing transvaginal sonographic examination and follow up until delivery. **Results** : The outcome was classified into two groups : firstly, 135 (60.3%) nonviable pregnancies including cases of blighted ovum 52 (23.2%), embryonic or fetal demise 31 (13.8%), complete abortion 28 (12.5%), incomplete abortion 13 (5.8%), ectopic pregnancies 7 (3.1%) and molar pregnancies 4 (1.8%); secondly, 89 (39.7%) viable pregnancies. Of the viable group, 8 (9.0%) eventually aborted spontaneously, and 81 (91.0%) continued their pregnancies beyond 20 weeks, in which 69 (85.2%) delivered at term and 12 (14.8%) ended in preterm births. The obstetric complications were comparable. **Conclusion** : The majority of pregnancies with first trimester threatened abortion were nonviable and transvaginal sonography was useful in clinical management. (Thai J Obstet Gynaecol 1995; 7:1-7.)*

Key words : threatened abortion, prognosis, transvaginal sonography

The term threatened abortion is a clinical description that indicates rather minimal bleeding of intrauterine origin, possibly associated with mild cramps in the absence of cervical dilatation at any time during the first 20 weeks of gestation.[1] It is a common complication that occurs in one out of four or five pregnant women. Despite efforts to alter the outcome, approximately one half of these pregnancies will eventually abort.[1] Furthermore, an increased risk of suboptimal pregnancy outcome in the form of preterm delivery, low birth weight and perinatal deaths was also noted.[2,3]

Clinical management depends on whether or not the embryo is alive. The treatment of choice for a nonviable pregnancy is uterine evacuation while the potentially viable gestation,

on the other hand, are expectantly observed. Sonographic evaluation of pregnancy is currently used to differentiate between a viable pregnancy and a pregnancy that inevitably will end in spontaneous abortion. Consequently, early diagnosis and management can be given to the patient with such a complication in the first trimester. Additionally, pregnancy outcome may be predicted in those patients with sonographically confirmed viable pregnancies.

This prospective study was conducted to evaluate the prognosis of pregnancies complicated with first trimester threatened abortion.

Materials and Methods

During a 16 month period between June 1, 1992 and September 30, 1993, a total of 224 pregnant women with clinical diagnosis of threatened abortion were admitted to the Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University. All the patients in the prospective study had uterine bleeding in the first trimester of pregnancy with or without lower abdominal discomfort deemed significantly by the physician. All patients met the following criteria of regular menstruation with certain date, positive urine pregnancy test or positive serum hCG, no serious medical complications and were able to attend follow up clinic until abortion or delivery at this university hospital. Unwanted pregnancy and twins gestation were excluded from the study.

Transvaginal sonography was performed by a single examiner with an Aloka model SSD 650 scanner with vaginal probe frequency of 5 MHz 90 degree. Sonographic findings were primarily classified as viable, nonviable pregnancy and inconclusive. Criteria for viable pregnancy included positive embryonic or fetal echo with evidence of fetal heart beats. Criteria for nonviable pregnancy included anembryonic pregnancy (blighted ovum) as defined by Nyberg's criteria[4], embryo or fetus with crown rump length more than 6 mm., or gestational age more than 7 weeks without demonstration of fetal heart motion, complete abortion, incomplete abortion, and other abnormal pregnancy such as molar and ectopic pregnancy. Inconclusive cases were defined as pregnancy without fetal echo but the conditions did not fulfill the criteria for diagnosis of blighted ovum i.e, mean sac diameter less than 25 mm, and undemonstrable fetal heart motion in a fetus with less than 7 weeks' gestation or less than 6 mm in crown rump length.

The treatment for nonviable pregnancy was appropriately done by uterine evacuation. The only treatment for viable pregnancy was bed rest. Patients with inconclusive criteria were evaluated 1 week later and managed according to subsequent sonographic findings. The patient underwent sonographic examination weekly if bleeding continued, if otherwise normal, follow up was

extended throughout gestation until delivery was achieved. If pregnancy failed, the aborted material was subjected to histologic examination to confirm diagnosis. Information of the patient's profile, sonographic findings and pregnancy outcome were collected in the microcomputer program SPSS PC + for analysis.

Results

The mean age ± SD. of 224 patients was 26.9 ± 5.22 years, range 16-46 years. Eighty five per cent of the patients were between 20-34 years of age. The first and second gravidas constituted 57% and 38% respectively. The mean ± SD gestational age at diagnosis of threatened abortion and transvaginal sonography performed was 8.67 ± 2.04 weeks, range 5-12 weeks.

Initial transvaginal sonographic findings of pregnant women with first trimester threatened abortion are presented in Table 1. Viable pregnancy was detected in 77 patients (34.4%), whereas, the first examination in 18 (8.0%) was inconclusive because the comceptive products were too small to allow detection or exclusion of fetal life. In the remaining 129 patients (57.6%) sonographic diagnosis indicating unsalvageable gestation could be made at the first examination.

In the inconclusive group, subsequent sonographic examination 1 week later showed living fetuses in 12 patients, early embryonic demise, bligthted ovum, complete abortion and incomplete abortion in 1, 3, 1, and 1 patients respectively.

The prognosis of all patients with threatened abortion in the first trimester is presented in Table 2 and summarized in Diagram 1. Of 89 patients of the viable pregnancy group, 8 patients (9.0%) had a spontaneous

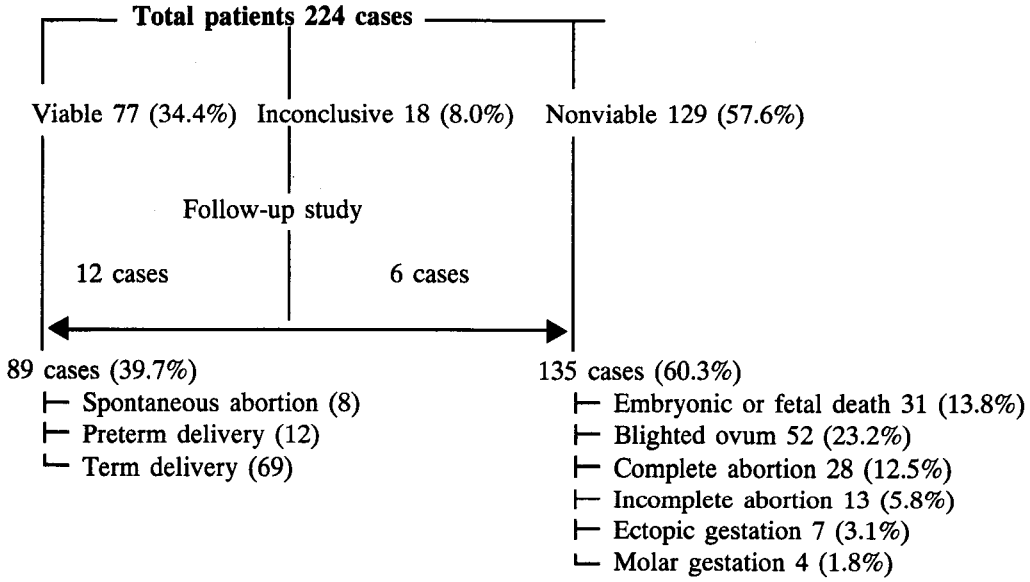
Table 1 ***Transvaginal sonographic finding at first examination in 224 patients with first trimester threatened abortion***

Sonographic findings	Number	Per cent
Viable pregnancies	77	34.4
Non viable pregnancies	129	57.6
Embryonic or fetal demise	30 (13.4 %)	
Blighted ovum	49 (21.9 %)	
Complete abortion	27 (12.1 %)	
Incomplete abortion	12 (5.4 %)	
Ectopic gestation	7 (3.1 %)	
Molar gestation	4 (1.8 %)	
Inconclusive	18 (8.0 %)	
Total	224	100.0

Table 2 ***Pregnancy outcome in 224 patients with first trimester threatened abortion***

Final diagnosis	Number	Per cent
Viable pregnancy	89	39.7
Spontaneous abortion	8	9.0
Continue pregnancy > 20 wks	81	91.0
- Preterm delivery (< 37 wks)	12	14.8
- Term delivery (≥ 37 wks)	69	85.2
Nonviable pregnancy	135	60.3
Embryonic or fetal demise	31	13.8
Blighted ovum	52	23.2
Complete abortion	28	12.5
Incomplete abortion	13	5.8
Ectopic gestation	7	3.1
Molar gestation	4	1.8
Total	224	100.0

Diagram 1 ***Prognosis of 224 patients with first trimester threatened abortion***



abortion, 81 continued beyond 20 weeks. Of the latter group, 12 (14.8%) had a preterm delivery, and 69 (85.2%) progressed to term.

In 135 patients of the nonviable group, blighted ovum was most commonly diagnosed, while ectopic and hydatidiform mole were incidentally diagnosed in 7 and 4 patients, respectively.

Late obstetric complications recorded in the viable group are

Table 3 *Late obstetric complications of the viable group (89 patients)*

Complication	Number	Per cent (of viable group)
Postterm (> 42 weeks)	2	2.2
Fetal growth retardation	6	6.7
Fetal death in utero	2	2.2
Preterm rupture of membranes	3	3.4
Chorioamnionitis	1	1.1
Pregnancy induced hypertension	4	4.5
Oligohydramnios	3	3.4
Placenta previa	2	2.2
Postpartum hemorrhage	1	1.1
Total	24	26.9

detailed in Table 3. Antepartum fetal deaths occurred in 2 patients. No intrapartum fetal death was found. Antepartum haemorrhage presented as placenta previa was found in 2.2% of the patients.

Discussion

Transvaginal sonography, at present, plays an important role in the management of pregnant women complicated by first trimester threatened abortion. Viable gestation can easily and accurately be differentiated from the nonviable ones with transvaginal sonographic examination.

The rate of abortion in this study was 60%, more than other reports in which the abortion rates were about 40-55%.[5-7] The higher risk of abortion in the present study is probably due to the tendency of this study population to delay seeking advice until the symptoms became more severe. Our patients with scanty bleeding frequently ignore the problem and do not come to the hospital, In these cases, continuation of viable pregnancy could generally be expected. Mantoni found that if the duration of bleeding was one to two days, the outcome in abortion was only 32%, whereas if bleeding lasted for three days or longer, the risk of abortion was significantly higher at 53%.[5] When the first bleeding episode began in weeks five to nine, the outcome in abortion was 35%. With later onset of bleeding, weeks 10 to 19, the abortion rate was 45%.[5] Patients of 35% in the present study had onset of bleeding after 9 weeks gestation.

This study showed that 95.5% of nonviable pregnancies could be dignosed by the first transvaginal sonography. Viable pregnancies and inconclusive group constituted 34.4% and 8%, respectively. These basic data

evidently indicated that transvaginal sonography is highly valuable in the management of pregnancy complicated with first trimester bleeding. Almost all nonviable gestations will eventually abort, but spontaneous expulsion may be delayed for weeks or even months after the onset of clinical symptoms.[8] This may lead to prolonged vaginal bleeding, infection, and patients' anxiety. Therefore, conservative treatment i.e. bed rest in these cases is avoidable. Early uterine evacuation spares the patient considerable expense, anxiety and discomfort.

Virtually all cases of molar pregnancy and ectopic pregnancy can be diagnosed early by the first sonographic examination. Incomplete abortion, by definition, should not be included in the study of clinically diagnosed threatened abortion. However, reports from other investigations indicated that incomplete abortion among cases diagnosed as threatened abortion by only clinical findings was found approximately 5-8%[5,9] as in this study (5.8%). Likewise, cases with complete abortion, 12.5% in this study, were also clinically diagnosed as threatened abortion despite closed cervical os and no history of conceptive product expulsion.

Blighted ovum was encountered in 38% in this series and was frequently found in the group of nonviable pregnancy. The major concern when an empty gestational sac is seen during threatened abortion is whether or not the pregnancy is viable. Viable pregnancies are managed conservatively, whereas, nonviable ones are treated by evacuation of the uterus. Criteria used for diagnosis of blighted ovum in this study were adopted from those of Nyberg et al[4] which represented the results of transabdominal investigation. It is generally accepted that, transvaginal scanning has a higher sensitivity e.g. earlier detection fetal heart activity and gestational sac, compared with transabdominal sonography.[10] Result of our study indicates that a single transvaginal sonography is useful in differentiating viable from nonviable gestational sacs. Mean sac diameter (MSD) of > 17 mm. that lacked an embryo or mean sac diameter of > 13 mm without visible yolk sac was found to be the most useful and reliable criterion for determining nonviability with 100% specificity and 100% positive predictive value.[11]

Despite the high resolution of transvaginal sonography, diagnosis could not be made in approximately 8% of patients with first trimester bleeding. The best approach in these cases is to repeat examination in 1 week and manage accordingly.

Whereas, the diagnosis of blighted ovum, complete or incomplete abortion and molar pregnancy can be made with accuracy, it is much more difficult to predict the outcome of the viable pregnancies. In the present study, despite demonstration of live fetus, 9% terminated as spontaneous abortion which was slightly higher than that reported by other investigators[5,6]

Preterm deliveries occurred in 14.8% of those continuing beyond 20 weeks. This figure was slightly higher than the incidence of preterm delivery in this institution (11.3%)[12] It is possible that first trimester threatened abortion increases the risk of preterm birth, however, further comparative study is needed to confirm this finding. Perinatal complications in the viable group were not found to differ significantly from the general obstetric population.

In summary, the majority of pregnancies with first trimester threatened abortion were nonviable and transvaginal sonography was useful in the clinical management. The outcomes of viable pregnancies were mostly normal, but preterm deliveries were relatively high.

References

1. Cunningham FG, Mac Donald PC, Gant NF, Leveno KJ, Gilstrap LC III. Williams obstetrics. 19th ed. East Norwalk : Appleton & Lange, 1993 : 675-676.
2. Batzofin JH, Fielding WL, Friedman EA. Effects of vaginal bleeding in early pregnancy on outcome. Obstet Gynecol 1984;63:515-518.
3. Funderburk SJ, Guthrie D, Meldrum D. Outcome of pregnancies complicated by early vaginal bleeding. Br J Obstet Gynaecol 1980;89:100-104.
4. Nyberg DN, Laing FC, Filly RA. Threatened abortion : sonographic distinction of normal and abnormal gestation sacs. Radiology 1986;158 :397-400.
5. Mantoni M. Ultrasound signs in threatened abortion and their prognostic significance. Obstet Gynecol 1985;65: 471-475.
6. Jouppila P. Clinical and ultrasonic aspects in the diagnosis and follow up of patients with early pregnancy failure. Acta Obstet Gynecol Scand 1980;59: 405.
7. Erikson PS, Philipsen T. Prognosis in threatened abortion evaluated by hormone assay and ultrasound scanning. Obstet Gynecol 1980;55:435.
8. Hertz JB. Diagnostic procedures in threatened abortion. Obstet Gynecol 1984;66:223.
9. Stabile I, Campbell S, Grudzinskas JG. Ultrasound assessment of complications during first trimester of pregnancy. Lancet 1987;1237-1240.
10. Timor-Tritsch IE, Rottem S. Pathology of the early intrauterine pregnancy. In : Timor-Tritsch IE, Rottem S, eds. Transvaginal sonography. 2nd ed. New York : Elsevier, 1991:314.
11. Tongsong T, Wanapirak C, Srisomboon J, Sirichotiyakul S, Sripolsuthiral T, Pongsatha S. Transvaginal ultrasound of the empty gestation sacs in threatened abortion. Int J Gynecol Obstet 1994;46: 297-301.
12. Annual Report 1993. Maternal fetal medicine. Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University. 1993:30.

Correlation of Uterine Blood Flow (UBL), Endometrial Thickness and Histopathology via Transvaginal Ultrasonoqraphy (TVS)

Damrong Tresukosol MD,
Boonchai Uerpairojkit MD,
Pairoj Witoonpanich MD, FRCOG,
Sukhit Paosavasdi MD.

Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Abstract : *Endometrial thickness and uterine Resistance Index of 140 women with abnormal uterine bleeding were studied by transvaginal ultrasonography and colour Doppler flow imaging. The results were reported and compared to histopathology obtained from dilatation and curettage. We found no abnormality if endometrium is less than 6 mm while the thickness more than 14 mm indicates endometrial histopathology. (Sensitivity, specificity and positive predictive value are 100, 46.6 and 27.9% respectively). This will reduce unnecessary diagnostic curettage to a level of 38.5%. Although thick endometrium may be a sign of pathological process, no morphological feature that are unique to a malignant disease has been identified. However intratumoral neovascularization with low impedance to blood flow was found and displayed by colour Doppler ultrasound in all the cancer cases. Both the endometrial thickness and intra tumoral blood flow were considered as a potential marker for endometrial carcinoma and may be useful for cancer detection in the future. (Thai J Obstet Gynaecol 1995;7:9-14.)*

Key Words : Uterine Blood Flow, endometrial thickness, histopathology, Trans-Vaginal Ultrasound (TVS)

With the introduction of transvaginal ultrasonography, thorough examination of the uterus can be easily accomplished.[1] Granberg et al.[2] reported that if endometrial thickness was less than 9 mm, no endometrial cancer would be diagnosed at curettage. Nasri et al.[3] agreed with Granberg's that an endometrial thickness of 5 mm was an appropriate cut off level for conservative management of patients with postmenopausal bleeding or in screening for endometrial cancer. Doppler flow imaging

may prove to be an important compliment to transvaginal sonography, improving its specificity as a screening test. Endometrial cancer shares with most malignant tumors the phenomenon of neovascularization. If there is increasing blood flow to endometrial tumors, the uterine arterial resistance should be lowered while benign endometrial proliferation should have normal uterine vascular flow pattern. The purpose of this study is to evaluate the correlation between endometrial thickness and Doppler waveform of the uterine circulation and the histopathological examination of the curetted specimens.

Materials and Methods

One hundred and forty women with abnormal uterine bleeding who had dilatation and curettage, between June 1, to March 31, 1993 were scanned with transvaginal ultrasound the day before the operation. The ultrasound examinations were performed by using the Aloka 680 SSD model. The vaginal probe was 5 MHz setting with high pass filter 100 Hz. The patient were divided into 3 groups;

Forty patients were under 40 years of age, 67 patients had perimenopausal bleeding with age range 40-50 years, and 38 patients had postmenopausal bleeding (cessation of menstrual period over 12 months interval). Patients with leiomyoma uteri were excluded.

Before ultrasonographic examination, the patient emptied the urinary bladder. The examination was performed with the patient in lithotomy position. The transducer was introduced into the posterior vaginal fornix, and the uterus was scanned longitudinally and transversely. Endometrial thickness was measured at the thickest part in the longitudinal plane. The measurement included both endometrial layers; that is, the measurement was performed between the two basal layers of the anterior and posterior uterine wall, also including the distended cavity. The poorly reflective layer surrounding the highly reflective endometrium was not included in the measurement. All structures were simultaneously examined. Transvaginal Doppler colour flow imaging of the uterine blood flow was identified in the transverse plane adjacent to the supravaginal portion of the cervix as described by Long et al.[6] The presence of intratumor vascularization with a low impedance blood flow were sought and recorded. The histopathologic diagnosis was used as "Gold standard".

All endometrial histology would be defined as "benign" except those who had endometrial hyperplasia, polyp or carcinoman which it would be classified as "pathologic".

Student unpaired *t-test* was used for statistical analysis.

Results

In Table 1 there were 129 patients whom ultrasound showed

normal uterus. The uterine cavity appeared as a linear central echo or small sonolucent area in the uterine cavity oriented in the cranio-caudal axis with a subendometrial halo. The histologic findings were 37 atrophic, 50 proliferative, 21 secretory, 13 hyperplasia and 11 cancers (Figure 1). In 10 cases endometrial tissues obtained from diagnostic curettage were insufficient for histologic diagnosis. These were classified as atrophic and benign even when it was not possible to obtain a histologic diagnosis. One had histological diagnosis of inactive endometrium with progestogenic effect and was considered as atrophic endometrium too. There were 31 patients with thick endometrium of more than 17 mm with or without fluid in the uterine cavity. 54.8% (17 cases) were histological proven hyperplastic or malignant. (Figure 1 and 2).

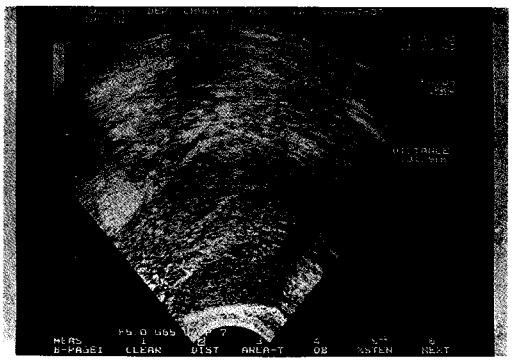


Fig. 1 The coronal plane showed multiple cystic cavitation. Pathologic finding was "cystic hyperplasia" or swiss cheese appearance.

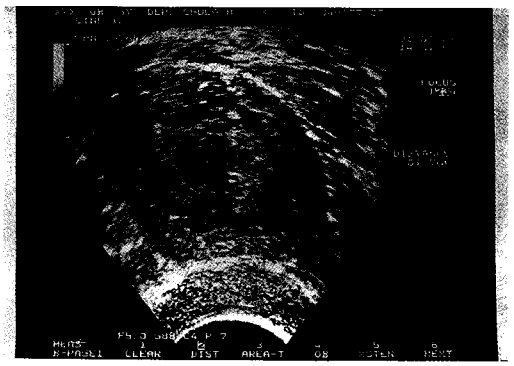


Fig. 2 This scan showed a postmenopausal uterus with a thick endometrium. The histologic finding revealed malignancy.

Table 1 *Ultrasound and histologic findings in 140 women with abnormal uterine bleeding*

Diagnosis	Ultrasound finding		Histologic finding	
	n	Endometrial Thickness (mms,)	D&C	n 140
Normal Uterus	129	8.7 (1-16)	Atrophic	37
			Proliferative	50
			Secretory	21
			Menstrual	8
			Hyperplasia	13
Cancer		33.8 (14-60)	Cancer	11

Mean endometrial thickness in atrophic, proliferative, secretory, menstrual, hyperplastic and malignant endometrium were 5.2 ± 3.8, 9.9 ± 6.1, 11.9 ± 6.3, 7.9 ± 6.4, 17.3 ± 7.3, and 20.5 ± 12.2 mm respectively. When compared uterine artery resistance index (R.I) with histologic subgroup, we found that women with endometrial cancer had lower uterine artery R.I. ($p < 0.05$) (Table 2). We were able to display all intratumoral vascularization with a low impedance to intratumor blood flow in the cancer cases Mean R.I. were 0.55 ± 0.06 which was significantly lower than that of uterine artery.

Discussion

Traditionally diagnostic curettage is one of the most common and acceptable operation performed for the peri and postmenopausal bleeding.[4] more recently transvaginal ultrasonographic examination has been considered by many centers as an alternative measure to dilatation and curettage for the patients[5] It is a relatively new technique with regard to its use for early detection of endometrial cancer. Few studies had been carried out using the endometrial thickness as the parameter to diagnose endometrial abnormality. Grandberg et al.[2] used vaginal ultrasound to study endometrial thickness in 205 postmenopausal women and found no abnormal endometrium if the thickness is ≤ 6 mm. They also suggested the cut off limit for endometrial abnormality to be ≤ 5 mm with a 87.3% positive predictive value 96% specificity and a 100% sensitivity to predict endometrial abnormality.[2] In our study malignancy was found with endometrial thickness of over 14 mm. This is in agreement with the study of Granberg[2] and Nasri[3]. Our mean endometrial thickness of cancer is 20.5 ± 12.2 mm. In women with a histopathologic diagnosis of atrophic endometrium, mean endometrial thickness was 5.2 ± 3.4 mm. We are quite confident that the difference between the thickness of atrophic and malignant endometrium could be easily revealed by ultrasound examination. If the thickness was more than 6 mm, 8 atrophic endometrium were found. When taking a cut off limit of 6 mm. for detection of endometrial abnormality, sensitivity, specificity and positive predictive value would be 100, 46.6 and 27.9% respectively (Table 3). Higher false positive cases in our study could be explained by recruitement of younger age group compared to previous studies. When Forty cases were less than 40 years of age. We believed that our preliminary data shows the potential benefit of transvaginal Doppler colour imaging in detection of endometrial melignancy. Though uterine vascular resistance index was not a reliable markerto differentiate benign from pathologic endometrium, but intratumor neovascularization hold promise. Our finding of intratumor blood flow of mean resistance

Table 2 *Impedance to uterine blood flow (as reflected by resistance index) in women with abnormal uterine bleeding and endometrial cancer*

Site of analysis of blood flow	Group	No of women	Resistance index Mean ± SE
Uterine arteries Right and left	Atrophic	37	604.1 ± 368.2
	Proliferative	50	564.9 ± 371.3
	Secretory	21	573.6 ± 362.0
	Menstrual	8	633.8 ± 394.6
	Hyperplasia	13	633.5 ± 383.6
	Cancer	11	314.6 ± 380.2 *(P<0.05)*
Within tumour	Cancer	5	549± 55.6 *(p < 0.5)*

Table 3 *Endometrial thickness and pathologic finding*

		Pathology		
		Abnormal	**Benign**	
Endometrial	> 7 mm	24	62	86
Thickness	≤ 6 mm	-	54	54
		24	116	

Sensitivity = 100 %
Specificity = 46.6 %
Positive predictive value = 27.9 %
Negative predictive value = 100 %

index 0.55 ± 0.06 may be one of the sensitive markers of endometrial[8] cancer which could be demonstrated in all of the cancer cases. These results seemed acceptable as we could avoid unnecessary curettage for at least one third of the cases. When we combined the Doppler indices with the sonographic endometrial thickness image, we believed that the false positive rate for diagnosis of endometrial cancer would be lessen, thus increased specificity could be achieved.[7]

Summary

We used the transvaginal ultrasound examination in women

with abnormal uterine bleeding prior to conventional Dilatation and Curettage It was a simple, non invasive technique, convenient and accepted by all patients. The endometrial thickness of less than 6 mm. was compatible with atrophic endometrium, but that of more than 14 mm. suggested malignancy. A cut off value for endometrial abnormality of 6 mm yield a sensitivity of 100%, specificity of 46.6% and positive predictive value of 27.9% (Fisher exac test). When endometrial thickness was more than 14 mm. intratumor flow mean resistance indices should be used to reduce false positive rate.

Acknowledgement

The author wish to express sincere thanks for a great support given by Professor Matsuto Mochizuki, Director of ICMR and head of department of obstetrics and Gynecology, Kobe University school of Medicine and Dr. Masaki Deguchi, M.D. during the training provided by JSPS Cooporation Programmes with Southeast Asian Countries Under the Core University System in Japan.

References

1. Nasri MN, Coast GJ. Correlation of ultrasound findings and endometrial histopathology in postmenopausal women. Br J Obstet Gynecol 1989;96: 1333-1338.
2. Granberg S, Wikland M, Karlsson B, Norstrom A, Friberg LG. Endometrial thickness as measured by endovaginal ultrasonography for identifying endometrial abnormality. Am J Obstet Gynecol 1991;164:47-52.
3. Nasri MN, Shepherd JH, Setchell ME, Lowe DG, Chard T. The role or vaginal scan in measurement of endometrial thickness in postmenopausal women. Br J Obstet Gynecol 1991;98:407-415.
4. Grimes DA. Diagnostic dilatation and curettage: a reappraisal. Am J Obstet Gynecol 1982;142:1-6.
5. MacKenzie IZ, Bibbly JG. Critical assessment of dilatation and curettage in 1029 women. Lancet 1978;2:566-568.
6. Long MG, Boultbee JE, Begent RHJ, Hanson ME. Doppler time velocity waveform studies of the uterine artery and uterus. Br J Obstet Gynecol 1989; 96:588-593.
7. Bourne TH, Campbell S, Whitehead MI, Royston P, Steer CV, Collins WP. Detection of endometrial cancer in postmenopausal women by transvaginal ultrasonography and colour flow imaging. BMJ 1990 Aug;301:369-370.

Effect of Citrate, Tris and Earle's Salt Solutions in Cryopreservative Media on the Percentage of Postthaw Motility and Cryosurvival Rate of Human Sperm

Anek Aribarg FRCOG,
Nares Sukcharoen MD,
Yenchit Chanprasit BSc,
Ken Boonmau.

Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Abstract : *The effects of citrate, Tris, and Earle's salt solution in cryopreservative media on the postthaw motility and cryosurvival rate of human sperm were compared. Each of eighty two ejaculates from donors and husbands was divided into three aliquots. Each aliquot was mixed with one of the three different media : 1) Citrate-egg yolk-glycerol, 2) Tris-egg yolk-glycerol, 3) Earle's salt solution-egg yolk-glycerol. The mixtures were vapour frozen, and stored at - 196°C. The percentage of sperm motility and cryosurvival rate were compared 30 minutes after thawing. Average percentage of sperm motility and cryosurvival rate after thawing for citrate, Tris, and Earle's salt solution were 26.4 ± 13.1%, 44.2 ± 15.1%, 25.7 ± 11.6%, 42.7 ± 14.6% and 27.8 ± 17.2%, 54.3 ± 15.6%, respectively. No statistically significant differences were found in sperm motility and cryosurvival rate obtained following thawing according to three media.(Thai J Obstet Gynaecol 1995;7:15-23.)*

Keywords : citrate / tris / Earle's salt solution, cryopreservative media, postthaw motility, cryosurvival rate.

The freezing and thawing of human sperm is undoubtedly associated with a decrease in sperm quality. The degree of impairment, however, is dependent on several factors. A major influence on cryosurvival of human sperm are the rate of freezing[1] and the nature of cryopreservative medium.[2] Currently, several types of cryopreservative media are employed with human sperm. Glycerol was firstly introduced[3] and due to its technical simplicity is still the most widely used cryoprotectant for freezing human sperm. Different studies have revealed the necessity[4-5] and the efficiency of glycerol compared to other cryoprotectants.[6-8] The addition of extenders, such as egg yolk and buffer systems, further provides a

better cryosurvival and motility.[6,9-13]

A more complex medium containing citrate-egg yolk-glycerol later was reported to result in higher human sperm cryosurvival when compared to glycerol alone.[9,14-15] Tris (hydroxymethyl) aminomethane (Tris)-egg yolk-glycerol was a traditional media developed and noted optimal results when freezing bovine sperm.[16] Recently, Earle's salt solution has been used in several different tissue culture systems, but it has never been used as a buffer system in cryopreservative media of human sperm.

Unfortunately, no comparative study has been conducted using all of these buffer systems to determine which cryopreservative medium is the best for postthaw motility and cryosurvival rate of human sperm. Therefore, the present study was conducted to compare the cryopreservative efficiencies of these three cryoprotective buffer systems.

Materials and Methods

To determine the optimal cryopreservative media for freezing human sperm, three cryopreservative buffers were used to freeze semen samples. Samples were collected from 82 fertile donors and patients attending infertility clinic at Chulalongkorn Hospital by masturbation into sterile glass containers after 3-5 days of sexual abstinence. Each sample was allowed to liquefy in room temperature for 30 minutes. The semen was examined to determine the sperm motility according to WHO guideline.[17] Following a prefreeze semen analysis, each semen sample was divided into three portions. Each portion was mixed with one of the media and cryopreserved as described below. Samples were thawed 1 week after freezing and analyzed for the percentage of postthaw sperm motility and sperm cryosurvival rate 30 minutes after thawing by an experienced technician. The sperm cryosurvival rate was calculated from the percentage of postthaw motility / the percentage of prefreeze motility x 100. Estimates were made in triplicate.

Preparation of Cryoprotective media

The following three buffer systems were prepared as cryopreservative media for human sperm : 1) citrate-egg yolk-glycerol : sodium citrate 70% (v:v), fresh egg yolk 20% (v:v), glycerol 10% (v:v), and Kanamycin sulfate 0.05 mg/L; 2) Tris-egg yolk-glycerol : Tris (hydroxymethyl) amino-methane 70% (v:v), fresh egg yolk 20% (v:v), glycerol 10% (v:v), and Kanamycin sulfate 0.05 mg/L ; 3) Earle's salt solution-egg yolk-glycerol : Earle's salt solution 70% (v:v), fresh egg yolk 20% (v:v), glycerol 10% (v:v), and Kanamycin sulfate 0.05 mg/L. The detailed composition of three buffer Systems were shown in Table 1 Sterile glassware, vials and pipettes were used and care was taken to avoid contamination of media. Eggs were purchased farm-fresh.

Table 1 *The detailed composition of thrce buffer systems*

Sodium citrate solution

Sodium citrate	7.25 g. in 250 ml. water
Glucose	13.65 g. in 250 ml. water
Fructose	13.65 g. in 250 ml. water

Earl's salt solution

Substance	M.M	g/L
$CaCl_2$. $2H_2O$	1.75	0.2649
KCl	5.37	0.004
$MgSO_4$. $7H_2O$	0.89	0.220
NaCl	116.34	6.800
NaH_2PO_4. $2H_2O$	1.02	0.1583
Glucose	5.55	1.0
Phenol Pred	0.048	0.017
$NaHCO_3$	10.00	0.85

Tris solution

Tris	30.28	g.
Citric acid	17.00	g.
Fructose	12.50	g.
Demineralized water	920	ml.

Freezing Technique

Each medium was added slowly over a 10 to 15 minute period to a portion of semen sample. Samples with a sperm density > 60 x 10^6 / ml were diluted at a ratio of 1:1 with the cryopreservative medium. Those with a density < 60 x 10^6 were diluted at a ratio of 3:1. The mixtures were packaged in 0.5 - ml plastic straws and then ends were sealed with polyvinyl cement. The straws were frozen by placing the straws individually on a horizontal rack situated 3cm above the liquid nitrogen (static vapor freeze at - 120°C). After 10 minutes, the straws were plunged into liquid nitrogen for storage at - 196°C.

Thawing Technique

The straws were thawed by removing them from the liquid nitrogen container and keeping them at room temperature (30°C) for 30 minutes.

Statistical Analysis

The sperm motility and cryosurvival rate were analyzed by two-

way analysis of variance (ANOVA), using the SPSS/PC+ statistical package (SPSS Ins., Chicago, USA) with respective to the cryopreservative media and the identity of the semen. The differences of $p<0.05$ were considered to be significant.

Results

The results are summarized in Table 2. Semen preserved with Earle's salt solution-egg yolk-glycerol showed higher cryosurvival than semen preserved with the rest of cryopreservative media, although the differences among the three media were not significant after thawing.

Table 2 ***Effect of citrate, Tris, and Earle' salt solution in cryopreservative media on the percentage of postthaw motility and cryosurvival rate of human sperm.***

Cryopreservative Buffers	Prefreeze Motility (%)	Postthaw Motility (%)	Cryosurvival Rate (%)
Citrate	58.6 ± 12.5 (35 - 80)	26.4 ± 13.1 (10 - 55)	44.2 ± 15.1 (28.6 - 68.7)
Tris		25.7± 11.6 (10 - 50)	42.7 ± 14.6 (28.6 - 62.5)
Earle's Salt Solution		27.8 ± 17.2 (10 - 60)	54.3 ± 15.6 (28.6 - 75.0)

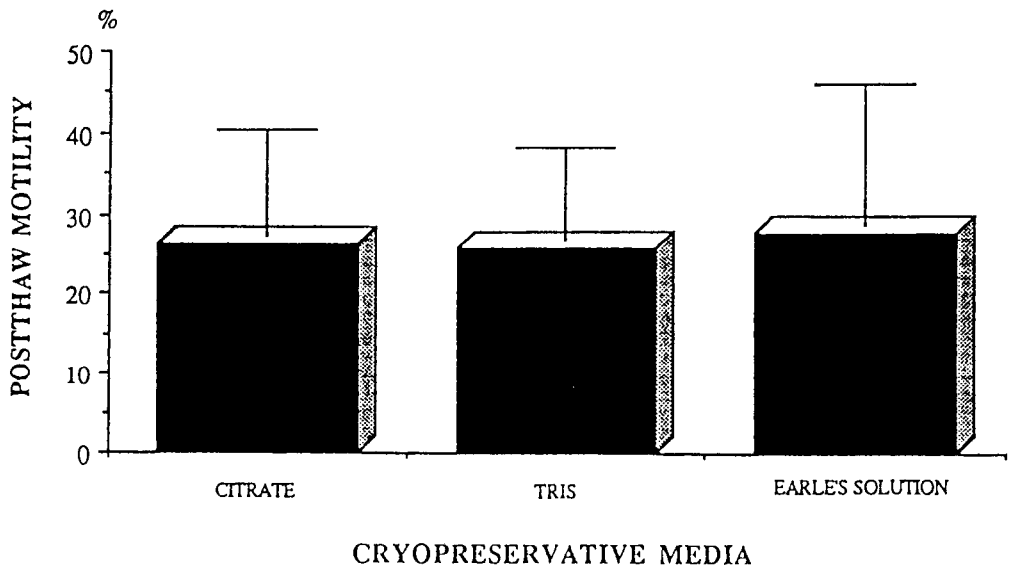


Fig. 1 Effect of Cryopreservative Media on Postthaw Motility

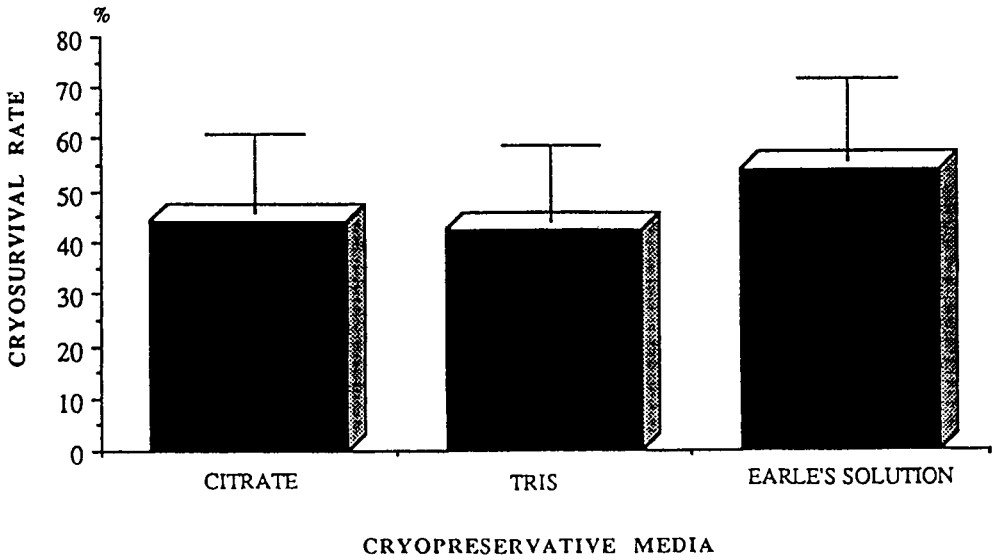


Fig. 2 Effect of Cryopreservative Media on Cryosurvival Rate

The percentage of postthaw motility and cryosurvival rate of cryopreserved sperm frozen in three different cryopreservative media are shown in Figure 1 and Figure 2, respectively. There were no statistical differences in the percentage of postthaw motility and cryosurvival rate of the cryopreserved sperm when they were frozen in three media.

Discussion

The freezing of sperm is a long-established technique and has been the subject of study for many years. Freezing of human sperm without a cryopreservative media results in severe damage to the sperm cells. Damage to the sperm cells during the freezing procedure have been demonstrated with transmission electron microscopy. When no cryopreservative medium is used the acrosome is found to be ruptured and their fine structure has been lost. Therefore, in all experiments on sperm freezing some type of cryopreservative medium has to be added to the ejaculate before freezing. Glycerol has been the main component in these media. Much efforts have been made to reduce this impairment in order to recover as many as possible progressively motile and morphologically normal sperm such as the addition of egg yolk and buffer systems to the cryopreservative media and freezing rate studies.[18]

A number of different cryopreservative media have been described for the conventional cryopreservation of human sperm based on the use of glycerol as the principal, permeable cryoprotectant in the presence or absence of extenders, which

include citrate, Tris, or other solutions, often in the presence of nonpermeable macromolecules such as egg yolk or albumin. It is possible that a medium containing egg yolk offers extra protection when freezing rates cannot be strictly controlled nor set at the optimal rates.[19]

Lipoproteins contained in the egg yolk reportedly protect sperm against cold shock[6,20], but may provide little protection during the periods of extracellular and intracellular ice formation. Cryoprotectants are required during these periods to protect sperm from the damaging effects of increasing salt concentrations and ice crystal formation. Comparative studies evaluating cryoprotectants are available[6,12], which allow several general conclusions

(1) A final concentration of glycerol between 5 and 10% with an equilibration time of 0-15 minutes at ambient temperature produces optimal results. The concentration of glycerol required for optimal survival of human sperm following cryopreservation is dependent on the type of medium used for freezing.[5]

(2) Common protocols include the direct addition of glycerol and the use of combinations of glycerol and extenders such as Tris in egg yolk buffer, usually added to semen at a 1:1 ratio. In order to minimize semen dilution inherent in the use of extenders, which will decrease the motile sperm available per insemination dose, the addition of 3 parts semen to 1 part glycerol plus extender can be employed.

Postthaw motility was found to be more highly correlated with pregnancy rates following therapeutic insemination of cryopreserved semen than prefreeze seminal quality (seminal volume, total sperm count, motility, morphology), postthaw motile sperm per inseminate or decrease in motility during cryopreservation.[21,22] Therefore, postthaw motility may be used as a clinical marker of freezing quality following observance of a significant correlation ($r = 0.98$) between postthaw motility and sperm with intact heads.[8] Although motility is not completely related to fertilizing capacity, it is generally accepted to be a sensitive parameter for evaluating freeze-thawing success.[23] Cryosurvival rate is also a sensitive parameter to assess the efficacy of cryopreservative process.

Freezing of the sperm after addition of a cryopreservative media, these solutions result in a much better preserved motility. In this study, three cryopreservative media have been compared under uniform conditions. Semen preserved with Earle's salt solution-egg yolk-glycerol showed higher cryosurvival rate and postthaw sperm motility than semen diluted with citrate-egg yolk-glycereol or Tris-egg yolk-glycerol, although the differences among these media were not significant. Previous studies using cryopreservative media for sperm cryopreservation has shown encouraging results and good postthaw motility of the spermatozoa has been ob-

tained.[24]

The choice of a cryopreservative medium for sperm should based on its ability to maintain the integrity and function of the cell during the freeze and thaw process. In the present study, we have shown that the percentage of postthaw sperm motility and cryosurvival rate were similar in all treatment groups.

The liquid nitrogen vapour technique introduced by Sherman in the early 1960s is still widely employed. This procedure involves sample exposure to liquid nitrogen vapours (-120°C) with or without the inclusion of a intermediate hold, typically at or near 4°C. the procedure is conducted without seeding, involves cooling rates of the order of 10-25°C/min depending upon the container and volume. Controlled-rate freezing (CRF) has gained popularity with increased equipment availability but has not yet proven superior for the cryopreservation of human semen. Theoretical advantages associated with the use of CRF include a more precise and reproducible cooling rate, which should lead to more effective, uniform dehydration and the prevention of intracellular ice crystal formation.[13] Although several investigators have described computer-controlled freezing methods as preserving sperm quality better than vapour freezing methods as preserving sperm quality better than vapour freezing[7,25-27], there is still controversy in the literature about its beneficial effect[10,13,28], at least for human sperm. On the other hand possible differences in cryopreservation efficiency have to be weighed against the differences in costs, time of execution and practical implication. In our hospital, liquid nitrogen vapour technique still has been used for sperm cryopreservation because it is rapid and relatively inexpensive.

The increasing risk of transmission of HIV and the concomittant risk of acquired immunodeficiency syndrome (AIDS), has now made the use of fresh donor semen unacceptable. The reported case of HIV being transmitted to recipients of fresh donor semen in a donor insemination (DI) programme in Australia underlines the risk involved.[29] Recently recommendations by the American Association of Tissue Banks and Centers for Disease Control that frozen semen be used for therapeutic insemination donor (TID) to decrease the likelihood of disease transmission[30], have resulted in renewed interest in developing better procedures and cryopreservative media for freezing human semen. Scant attentions have been given to the presence of interactions among various cryopreservation methodologies and cryopreservative media in developing optimal procedures for freezing human sperm. In contrast, the importance of these interactions in developing effective methods for freezing bovine semen is well known.

In conclusion, semen preserved with Earle's salt solution-egg yolk-glycerol showed higher cryosurvival than semen preserved with the rest of

cryopreservative media, although the differences among the these media were not significant after thawing. Further study is warranted to determine the effect of these cryopreservative media on fertilizing ability.

References

1. Freund M, Wiederman J. Factors affecting the dilution freezing, and storage of human semen. J Reprod Fertil 1966;11:1-17.
2. Friberg J, Gemzell C. Sperm freezing and donor insemination. Int J Fertil 1987;22:148-154.
3. Polge G, Smith AU, Parkes AS. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 1949;164:666-669.
4. Critser JK, Huse-Benda AR, Aaker DV, Arneson BWA, Ball GD. Cryopreservation of human spermatozoa. III. The effect of cryoprotectants on motility. Fertil Steril 1988;50:314-320.
5. Hammitt DG, Walker DL, Williamson RA. Concentration of glycerol required for optimal survival and in vitro fertilizing capacity of frozen sperm is dependent on cryopreservation medium. Fertil Steril 1988;49:680-687.
6. Mahadevan M, Trounson AO. Effect of cryoprotective media and dilution methods on the preservation of human spermatozoa. Andrologia 1983;15:355-366.
7. Serafini P, Marrs RP. Computerized staged-freezing technique improves sperm survival and preserved penetration of zona-free hamster ova. Fertil Steril 1986;45:854-858.
8. Serafini P, Hauser D, Moyer D, Marrs RP. Cryopreservation of human spermatozoa : correlations of ultrastructural sperm head configuration with sperm motility and ability to penetrate zona-free hamster ova. Fertil Steril 1986;46:691-695.
9. Harrison RF, Sheppard BL. A comparative study in methods of cryoprotection of human semen. Cryobiology 1979;17: 25-32.
10. Thachill JV, Jewett MAS. Preservation techniques for human semen. Fertil Steril 1982;37:100-103.
11. Heath E, Jeyendran RS, Perez-Palaez M, Sobrero AJ. Ultrastructiural categorization of human sperm cryopreserved in glycerol and in TESTCY. Int J Androl 1985;8:101-110.
12. Prins GS, Weidel L. A comparative study of buffer systems as cryoprotectants for human spermatozoa. Fertil Steril 1986;46:147-149.
13. Wolf DP, Patton PE. Sperm cryopreservation: State of art. J In Vitro Fertil Embryo Transfer 1989;6:325-327.
14. Behrman SJ, Ackerman DR. Freeze preservation of human sperm. Am J Obstet Gynecol 1969;103:654-661.
15. Matheson GW, Carlborg L, Genzell C. Frozen human semen for artificial insemination. Am J Obstet Gynecol 1969;104:495-501.
16. Graham EF, Crabo BG, Brown KI. Effects of some zwitter ion buffers on the freezing and storage of spermatozoa. I. Bull J Dairy Sci 1972;55:372-378.
17. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 2nd edition. Cambridge University Press, Cambridge 283-350.
18. Watson PF. The preservation of semen in mammals. In : Finn CA, ed. Oxford Reviews of Reproductive Biology. Vol.1 Cambridge : Oxford University press 283-350.
19. Trounson AO, Mahadevan M, Wood J, Leeton JF. Studies on the deep freezing and artificial insemination of human semen. In: Richardson D, Joyce D, Symonds M, eds. Frozen Human Semen. London : RCOG 1979:173-181.
20. Bolanos JR, Overstreet JW, Katz DF. Human sperm penetration of zonafree hamster eggs after storage of the semen

for 48 hours at 2°C to 5°C. Fertil Steril 1983;39:536-541.
21. Mayaux MJ, Schwartz D, Czyglik F, David G. Conception rate according to semen characteristics in a series of 15,364 insemination cycles: results of a multivariate analysis. Andrologia 1985;17:9-16.
22. David G, Czyglik F, Mayaux MJ, Schwartz D. The success of AID and semen characteristics: study on 1489 cycles and 192 ejaculates. Int J Androl 1980;3:613-619.
23. Cross NL, Hanks SE. Effects of cryopreservation in human sperm acrosomes. Hum Reprod 1991;6:1279-1283.
24. Weidel L, Prins GS. Cryosurvival of human spermatozoa frozen in eight different buffer systems. J Androl 1987; 8:41-47.
25. Taylor PJ, Wilson J, Laycock R, Weger J. A comparison of freezing & thawing methods for the cryopreservation of semen. Fertil Steril 1982;37:100-103.
26. McLaughlin EA, Ford WCL, Hull MGR. A comparison of the freezing of human semen in the uncreculated vapoor above liquid nitrogen and in a commercial semi programmable freezer. Hum Reprod 1990;5:724-728.
27. Ragni GR, Caccamo AM, Dalla Serra A, Guercilena S. Computerized slowstaged freezing of semen from men with testicular tumors or Hodgkin's disease preserves sperm better than standard vapor freezing. Fertil Steril 1990;53: 1072-1075.
28. Verheyen G, Pletinox I, Van Steirteghem. Effect of freezing method, thawing temperature and postthaw dilution/washing on motility (CASA) and morphology characteristics of high-quality human sperm. Hum Reprod 1993;8:1678-1684.
29. Stewart GJ, Tyler JPP, Cunningham AL, Barr JA, Driscoll GL, Gold J, Lamont BJ. Transmission of human T-cell lymphotropic virus type III (HTLV-III) by artificial insemination by donor. Lancet 1985;2:581-584.
30. Sherman JK. Current status of clinical cryobanking of human semen. In: Paulson JD, Negro A, Lucena E, Martini L, eds. Andrology : Male Fertility and Sterility. New York : Academic Press 1986:517-538.

Comparison of Sperm Motility and Sperm Recovery Rate After 40%/80% and 50%/100% Discontinuous Percoll Gradients Preparation

Nares Sukcharoen MD.

Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Abstract : *Two discontinuous Percoll gradients (40%/80% and 50%/100% Percoll gradients) were assessed for efficacy of selection of motile sperm from 55 normozoospermic semen samples. Each semen sample was assessed for basic semen parameters before and after preparation by both techniques. The preparation of sperm on both two step Percoll gradients allowed about one third of the progressively motile sperm to be recovered. Sperms prepared by the 50%/100% Percoll gradients had higher percent progressive motility and percent normal morphology but there were no statistically significant differences between both techniques. Sperm preparation by 40%/80% Percoll gradients seems to be better than 50%/100% Percoll gradients because total sperm number, total motile sperm number, and total sperm recovery rate after preparation by 40%/80% Percoll gradients were greater than 50%/100% Percoll gradients significantly. (Thai J Obstet Gynaecol 1995;7:25-32.)*

Key Words : Discontinuous Percoll gradients preparation/ Normal semens

Short Title : Discontinuous Percoll gradients for normal semens

Percoll, a medium composed of colloidal silica particles coated with polyvinyl-pyrrolidone, has been found to improve the isolation of motile spermatozoa, generally free of contamination from other seminal constituents. Percoll gradients was found to return a high proportion of functional sperm. The buoyant density apparently protects the sperm from the trauma of centrifugation.[1] The use of Percoll to create buoyant density gradients in the preparation of human was firstly described using continuous self-generating gradients produced by high-speed centrifugation.[2] The production of continuous Percoll gradients is time-consuming and requires a high-speed centrifuge; therefore, a two-step discontinuous gradient which is more simple to prepare was intro duced.[3] Subsequently,

discontinuous Percoll gradients has been widely used in many laboratories for separating human sperm. This method is suitable for sperm preparation because it has the advantage of being a comparatively rapid and effective method.

The two-layer Percoll density gradients concentration that have been used and recommended in many reports are 40%/80% Percoll gradients[4-7] and 50%/100% Percoll gradients.[8,9] No comparative study of sperm morphology, sperm motility and sperm recovery rate after both sperm preparation techniques has been reported. This study was conducted to compare sperm morphology, sperm motility and the recovery of motile sperm form normozoospermic semen samples.

Materials and Methods

Semen Collection

This study was based upon 55 samples collected from the 45 volunteers, all of whom possessed normal semen profiles according to WHO guideline.[4] The semen samples were obtained by masturbation after at least 48 hours of abstinence and collected into sterile plastic containers. After liquefaction, samples were assessed for conventional parameters (volume, morphology, motility, sperm concentration, and round cell concentration) using the procedures laid down by the World Health Organization.[4] The remainder was divided into equal portions and then recovered by the 40%/80% and 50%/100% Percoll centrifugation separation technique.

Percoll Gradient Preparation

Isotonic Percoll was created by supplementing 10 ml of 10 x concentrated medium 199 (Flow Laboratories, Irvine, UK) with 300 mg bovine serum albumin (BSA), 3 mg sodium pyruvate and 0.37 ml sodium lactate syrup, and adding 90 ml of Percoll (Pharmacia, Uppsala, Sweden). This preparation was designated 100% percoll[10] and subsequently diluted to 40%, 50%, 80% with medium BWW.[11]

40%/80% Percoll Gradient Preparation

For each semen sample, a two-layer Percoll discontinuous gradient comprising 3 ml 80% Percoll overlaid with a further 3 ml 40% Percoll was prepared in a 15-ml(17x120 mm) sterile conical test tube.

50%/100% Percoll Gradient Preparation

For each semen sample, a two-layer Percoll discontinuous gradient comprising 3 ml 100% Percoll (isotonic Percoll) overlaid with a further 3 ml 50% Percoll was prepared in a 15-ml(17x120 mm) sterile conical test tube.

Each equal portion of remaining semen samples after initial assessment was carefully layered on the top of each prewarmed (37° C) gradients.

Centrifugation and Collection of Sperm from Gradients

The Percoll gradients were centrifuged at room temperature in a swinging bucket rotor at 500g for 20 minutes and the seminal plasma was then discarded and the cells collected into two fractions derived from (1) the base of the 100% Percoll fraction (100% fraction), (2) the base of the 80% Percoll fraction (80% fraction). The cells from each fraction were resuspended in 5 ml of BWW, centrifuged at 500g for 5 minutes and finally resuspended in approximately 0.5 ml BWW.

Each sperm suspension from 80% fractions and 100% fractions were assessed for percent normal morphology, percent progressive motility, total recovered sperm number, total recovered motile sperm number.

The total sperm recovery rate was calculated as:

(Final recovered volume x Final sperm concentration) / (Initial semen volume x Initial sperm concentration)

The sperm motility recovery rate was calculated as:

(Final recovered volume x Final sperm concentration x Final motility) / (Initial semen volume x Initial sperm concentration x Initial motility)

And expressed as a percentage. The sperm motility recovery rate reflected the proportion of motile spermatozoa from the initial sample that were effectively recovered by this preparation method.

Statistical Analysis

Results are experssed as the mean ± standard error of means (SEM). The significance of differences between mean results of percent motile sperm, percent normal morphology, total sperm recovery rate, and motile sperm recovery rate from both techniques were compared by paired t-test. A probability of 0.05 was assumed to denote a significant difference. Statistical analyses were carried out using the STATVIEW programme on an Apple Mcintosh SE.

Results

The population of donors used in this study exhibited the mean semen characteristics shown in Table 1.

Comparison of the semen parameters measured before and after sperm preparation by both discontinuous Percoll gradients, the per cent progressive motility, per cent normal morphology after sperm preparation by both techniques increased significantly ($p<0.001$), but total sperm number, total motile sperm number and total round cell number reduced significantly ($p<0.001$). The preparation of sperm on both two-step Percoll gradients allowed about one-third of the progressively motile sperm to be recovered.

Sperms prepared by the 50%/100% Percoll gradients had better progressive motility and more normal morphology but the difference's were not statistically significant.

Table 1 *Semen parameters before discontinuous Percoll gradient preparation*

Semen Parameters	Mean ± SEM
Volume (ml)	3.3 ± 0.2
Normal Morphology (%)	36.7 ± 2.4
Progressive Motility (%)	69.1 ± 1.4
Semen Concentration (x 10^6/ml)	116.3 ± 11.2
Round Cell* Concentration (x 10^6/ml)	1.9 ± 0.3

*Round cells include polygonal epithelial cells from the urethral tract, spermatogenic cells, and leucocytes. As a general guide, a normal ejaculate should not contain more than 5 x 10^6 round cells/ml.

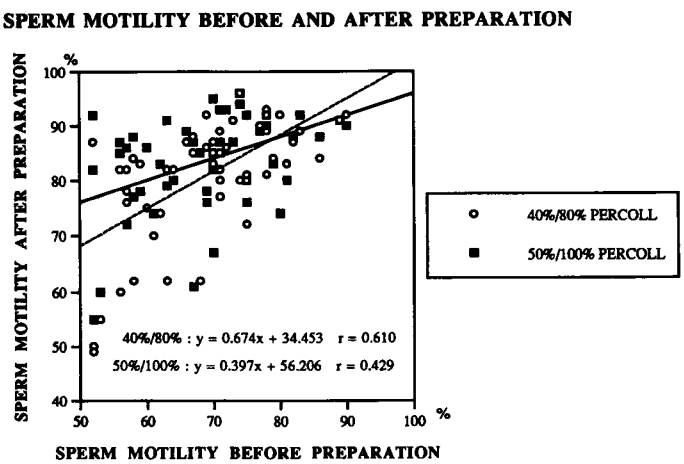


Fig. 1 Comparison of the percent progressive motility of sperm before and after sperm preparation by 40%/80% (open circles) and 50%/100% (solid squares) discontinuous Percoll gradients preparations. The correlation between percent progressive motility before and after sperm preparation by 40%/80% (broken line) and 50%/100% (solid line) Percoll gradients were demonstrated.

Figure 1 shows the per cent progressive motility of sperm before and after sperm preparation on both Percoll gradients. Linear regression analysis revealed a direct positive relationship between the percent progressive motility of sperm before and after sperm preparation by both techniques.

The total motile sperm recovery rate from final Percoll fraction was calculated based on the total number of sperm in the semen and the percent progressive motile sperm found in the initial semen and the in each fraction as in the formula shown above. The percentage of sperm recovered varied widely from person to person. In this study, total sperm number and total motile sperm number after prepared by 50%/100% Percoll gradients were less than 40%/

Table 2 *Comparison of the semen parameters measured before and after sperm preparation by two discontinuous Percoll gradients*

Semen Parameters	Before Preparation	After Preparation 40%/80% Percoll	50%/100% Percoll	
Normal Morphology (%)	36.7 ± 2.4	78.5 ± 2.4	81.6 ± 3.5	NS
Progressive Motility (%)	69.0 ± 1.4	80.8 ± 1.5	83.5 ± 1.2	NS
Total Sperm Number (x10^6)	96.2 ± 10.3	47.6 ± 5.0	37.8 ± 3.8*	
Total Motile Sperm Number (x10^6)	65.5 ± 6.8	38.4 ± 4.1	31.9 ± 3.5*	
Total Round Cell Number (x10^6)	0.95 ± 0.15	0.03 ± 0.19	0.03 ± 0.02	NS
Total Sperm Recovery Rate (%)		32.8 ± 4.8	23.9 ± 1.7*	
Motile Sperm Recovery Rate (%)		37.6 ± 5.4	28.9 ± 2.3	NS

* $p<0.05$, NS : No Statistical significance

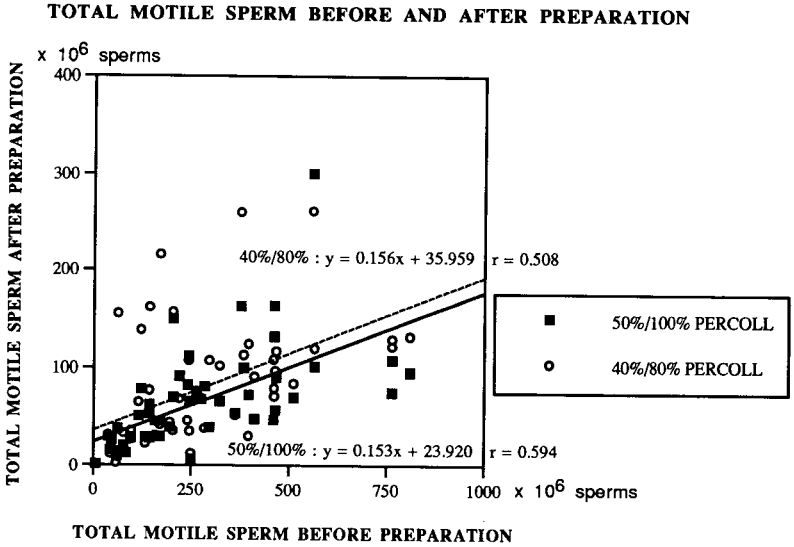


Fig. 2 Comparison of the total motile sperm number before and after sperm preparation by 40%/80% (open circles) and 50%/100% (solid squares) discontinuous Percoll gradients preparations. The correlation between total motile sperm number before and after sperm preparation by 40%/80% (broken line) and 50%/100% (solid line) Percoll gradients were demenstrated.

80% Percoll gradients significantly. Linear regression analysis revealed a direct positive relationship between the total motile sperm number before and after sperm preparation by both techniques. Figure 2 shows the total motile sperm number before and after sperm preparation on both Percoll gradients.

The total sperm number, total motile sperm number, and total sperm recovery rate were significantly hig-

her, when 40%/80% Percoll gradients were used. The motile sperm recovery rate from the 50%/100% Percoll gradients and the 40%/80% Percoll gradients were similar (p=0.0577), but considerably fewer motile sperms were recovered from 50%/100% Percoll gradients.

Discussion

A variety of methods have been used to separate motile sperm from semen, and each has both desirable and undesirable attributes. The ideal separation technique should: 1) be rapid, simple, and inexpensive, 2) recover most of motile sperms in the specimen. 3) result in no damage or physiologic alteration of the separated sperm, 4) remove dead sperm andother cells, including microorganisms, 5) remove toxic and bioactive substances, 6) process large volumes of semen, and 7) allow the final volume of the sperm suspension to be controlled. Depending on the application, these criteria differ in their importance.[7]

The Percoll gradient preparation has been demonstrated to yield good sperm recovery and final sperm motility[3,9,10,12-14] It is a very simple and convenient sperm preparation technique for selecting sperm with good fertilizing ability as evaluated by laboratory tests, having a longer survival[15], enhanced motility[9,15-17] and superior nuclear maturing[18]. The volume of the sperm suspensions after Percoll separation could be adjusted because both methods involve resuspension of a pellet of the desired volume. Therefore, discontinuous Percoll gradients have been widely used in many laboratories for separating human sperm and it seems to be the best method for sperm preparation.

In this study, the comparison of the efficacy of motile sperm selection by 50%/100% Percoll with that by 40%/80% was chosen because no comparative study of sperm morphology, sperm motility and sperm recovery rate after both sperm preparation techniques has been reported. The preparation of sperm on both two-step Percoll gradients allowed about one-third of the progressively motile sperm to be recovered. Both gradients gave a high yield of motile sperms. The progressive motility, normal morphology and the motile sperm recovery rate after preparation by both methods did not differ significantly. The statistically significant improvement of percent progressive motility indicated the validity of sperm selection by both methods of discontinuous Percoll gradients. The final samples were found to be fairly free of round cells (germinal cells and leucocytes).

Both Percoll gradients were very successful in eliminating morphologically abnormal sperm. In this study, the result was similar to the previous report[19], but the increase in normal forms was not shown in some reports.[18,20] These differences can be explained either by the lack of uniformity in the Percoll gradient

preparation technique, or by the classification used to describe morphology.

The results of this investigation also indicated a direct relationship between the percent progressive motility and total motile sperm number before and after preparation on discontinuous Percoll gradients. The total sperm number, total motile sperm number, and total sperm recovery rate were significantly higher, when 40%/80% Percoll gradients were used. The motile sperm recovery rate from both discontinuous Percoll gradients in this study was lower than previous reports.[10,12] Although the total motile sperms recovered from 40%/80% Percoll gradients had considerably higher number than 50%/100% Percoll gradients, the motile sperm recovery rate in the two fractions were not significantly different (p=0.0577)

Sperm preparation by 40%/80% Percoll gradients seems to be better than 50%/100% Percoll gradients because total sperm number and total motile sperm number after prepared by 40%/80% Percoll gradients were greater than 50%/100% Percoll gradients significantly. The sperm preparation by 40%/80% Percoll gradients warrant further investigation as a density gradient medium for the selection of motile human spermatozoa from poor semen samples, especially, oligozoospermia because it yielded higher total sperm number and total sperm recovery rate. However, centrifugation on both two-step Pecoll gradients are convenient and efficient ways to prepare normal morphology and motile sperm for assisted reproduction techniques from normo-zoospermic semen samples.

References

1. Aitken RJ. Assessment of sperm function for IVF. Hum Reprod 1988;3:89-95.
2. Braude PR, Bolton VN. The preparation of spermatozoa for in vitro fertilization by buoyant density centrifugation. In : Feichtinger W, Kemeter P, eds. Recent Progress in Human In Vitro Fertilization. Palermo: Cofese 1984:125-134.
3. Dravland JE, Mortimer D. A simple discontinuous Percoll fradient for washing human spermatozoa. IRCS Med Sci 1985;13:16-17.
4. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. Cambridge University Press : Cambridge, 1992.
5. Aitken RJ, West KM. Analysis of the relationship between ractive oxygen species production and leucocyte infiltration in fractions of human semen separated on Percoll gradients. Int J Androl 1990;13:433-451.
6. Ford WCL, McLaughlin EA, Prior SM, Rees JM, Wardle PG, Hull MGR. The yeild, motility and performance in the hamster egg test of human spermatozoa prepared from cryopreserved semen by four different methods. Hum Reprod 1992;7:654-659.
7. Drobnis EZ, Chang QZ, Overstreet JW. Separation of cryopreserved human semen using Sephadex columns, washing, or Percoll gradients. J Androl 1991;12: 201-208.
8. Aitken RJ, Buckingham D, West K, Wu FC, Zikopoulos K, Richardson DW. Differential contribution of leucocytes

and spermatozoa to the generation of reactive oxygen species in the ejaculates of oligozoospermic patients and fertile donors. J Reprod Fert 1992:94:451-462.

9. McClure RD, Nunes L, Tom R. Semen manipulation : improved sperm recovery and function with a two-layer Percoll gradient. Fertil Steril 1989;51:874-877.
10. Lessley BA, Garner DL. Isolation of motile spermatozoa by density gradient centrifugation in Percoll. Gamete Res 1983;7:49-761.
11. Biggers JS, Whitten WK, Whittingham DG. The culture of mouse embryos in vitro. In : Daniel JC Junior, eds. Methods in Mammalian Embryology. Freeman: San Francisco 1971:86-116.
12. Berger T, Marrs RP, Moyer DL. Comparison of techniques for selection of motile spermatozoa. Fertil Steril 1985; 43:268-273.
13. Forster MF, Smith WD, Lee WI, Berger RE, Karp LE, Stenchever MA. Selection of human spermatozoa according to their relative motility and their interaction with zona-free hamster eggs. Fertil Steril 1983;40:655-660.
14. Arcidiacono A, Walt H, Campana A. The use of Percoll gradient for the preparation of subpopulations of human spermatozoa. Int J Androl 1983;6: 433-445.
15. Guerin JF, Mathieu C, Lornage J, Pinatel MC, Boulieu D. Improvement of survival and fertilizing capacity of human spermatozoa in an IVF programme by selection on discontinuous Percoll gradients. Hum Reprod 1989;4:798-804.
16. Gellert-Mortimer ST, Clarke GN, Baker HWG, Hyne RV, Johnston WIH. Evaluation of Nycodenz and Percoll density gradients for the selection of motile human spermatozoa. Fertil Steril 1988;49: 335-341.
17. Akerlof E, Fredicson B, Gustafsson O, Lundin A, Lunell LO, Nylund L, Rosenberg L, Pousette A. Comparison between a swim-up and a Percoll gradient technique for the separation of human spermatozoa. Int J Androl 1987;10: 663-669.
18. Le Lannou D, Blanchard Y. Nuclear maturity and morphology of human spermatozoa selected by Percoll density gradient centrifugation or swin-up procedures. J Reprod Fertil 1988;84:551-556.
19. Van der Zwalmen P, Bertin-Segal G, Geerts L, Debauche C, Schoysman R. Sperm morphology and IVF pregnancy rate: comparison between Percoll gra-dients centrifugation and swim up procedures. Hum Reprod 1991;6:581-588.

Elevation of Serum Steroid Sulfatase Level in Gynecologic cancers

Teruo Sugawara[1],
Koichi Honke[2],
Akita Makita[2],
Seiichiro Fujimoto[1]

[1]*Department of Obstetrics and Gynecology and* [2]*Biochemistry Laboratory, Cancer Institute, Hokkaido University School of Medicine, Kita-ku North 15, West 7, Sapporo 060, Japan.*

Abstract : *Steroid sulfatase (STS) desulfates sulfated 3 β-hydroxysteroid family, such as, estrone sulfate, androstenediol sulfate, dehydroepiandrosterone sulfate and cholesterol sulfate. This enzyme possesses an important role in the converting the inactive steroid hormone to the active form. We have established an enzyme-linked immunosorbent assay (ELISA) of STS to measure the amount of the enzyme protein in sera. ELISA was performed by "Sandwich" method using a peroxidase conjugated anti STS IgG Fab' fragment. A range of STS was 10 to 1,500 ng per ml serum in this method. When gynecologic carcinomas were assayed by this ELISA, the serum STS level was significantly ($P<0.01$) elevated in endometrial carcinoma and ovarian carcinoma patients, as compared to that of normal women. Measurement of serum STS protein may be useful for clinical applications as a tumor marker. (Thai J Obstet Gynaecol 1995;7:33-40.)*

Key words : steroid sulfatase; gynecologic cancers; enzyme-linked immunoassay; ovarian cancer; endometrial carcinoma

Steroid sulfatase (E.C. 3.1.6.2.) is widely distributed in the mammalian tissues such as liver, ovary, testis, and uterine endometrium and is especially abundant in placenta[1]. This enzyme catalyzes desulfation all the 3 β-hydroxysteroid sulfates, including estrone sulfate, and pregnenolone sulfate. Sulfated form of the steroid hormones is inactive because of failure to bind to their receptors. The hydroxysteroid sulfates become active after desulfation by STS[2]. Sulfated steroid hormones which represent in blood as the major form of the steroid hormones acts as a reservoir or precursors of active hormones[3]. The development and growth of endometrial carcinoma[4], ovarian carcinoma[5] depends on estrogen. These observations suggest that STS is involved in the genesis and the

maintenance of steroid hormone depended on tumors such as endometrial carcinoma an ovarian carcinoma[6]. Some recent reports have demonstrated that STS activity was significantly higher in endometrial carcinoma tissue than normal endometrium[7]. It will be of value to know STS level in blood for elucidation of the tumor growth and diagnostic and prognostic meaning in steroid dependent carcinomas. However, STS activity level in blood is under detectable level by the methods so far developed. In this paper, we have developed an enzyme-linked immunosorbent assay (ELISA) of serum STS to measure an amount of the enzyme protein in sera of patients with gynecological carcinomas.

Materials and Methods

Sera from normal subjects and patients. The normal control sera (69 women and 8 men) were obtained from healthy volunteers. The patients' sera prior to any treatment were from 30 patients with cervical carcinoma, 17 with endometrial carcinoma, 13 with ovarian carcinoma. All samples were kept frozen at - 20°C before analysis.

Material [7-^{3}H] dehydroepiandrosterone sulfate was purchased from New England Nuclear (Boston, USA); non labeled dehydroepiandrosterone from Sigma (St. Louis, USA); concanavaline A (Con-A) Sepharose, Blue-Sepharose, octyl Sepharose, Mono P, and PD-10 from Pharmacia (Uppsala, Sweden); pepsin, 2 mercaptoethylamine and Triton X-100 from Sigma (St Louis, USA); N-succinimidy l-6-maleimidohexanoate (EMCS) and peroxidase from Dojinkagaku (Kumamoto, Japan) and from Toyoby (Tokyo, Japan), respectively; microtiter-plate for ELISA from Nunk (Roskilde, Denmark); Centricon from Amicon (Danvers, USA); Tween 20 from Wako Jyunyaku (Tokyo, Japan). All other reagents are of reagent grade.

Purification of human steroid sulfatase. STS was purified from term human placenta, as previously described by Yen et al[8] with a slight modification, the human placenta was homogenized with three volumes of 10 mM Tris-HCl, pH 7.5 containing 0.05% Triton X-100. The homogenate was centrifuged at 10,000 x g for 30 min. The supernatant was discarded. The precipitate was dissolved in three volumes of 10 mM Tris-HCl, pH 7.5 containing 1% Triton X-100 and stirred. Following centrifugation at 10,000 x g for 30 min, the supernatant containing STS activity was subjected to sequential chromatographies on Con A Sepharose, Blue Sepharose, octyl Sepharose, and chromatofocusing on Mono P. Purification was monitored by assaying STS activity using [7^{3}H] dehydroepiandrosterone sulfate as described previously[9]. One unit (U) of the enzyme activity was defined as one nmol of the product per hour.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) and

2-mercaptoethanol was performed on 10% polyacrylamide gel as described previously by Laemmli[10]. The gel was stained using silver staining kit (Kanto Chemical, Tokyo, Japan).

Preparation of antibody against steroid sulfatase. Rabbit antisera against STS was produced by immunizing 3 time with the purified preparation (100 μ g each) emulsifed three times in Freund's complete adjuvant. The IgG was purified by ammonium sulfate precipitation and DEAE-cellulose chromatography. Immunoblot was carried out as described previously[11].

Conjugation of anti STS-IgG Fab' with peroxidase. This was carried out as described previously by Yoshitake[12]. Briefly, the IgG (32mg) was digested with pepsin (0.6 mg) followed by isolation of F(ab')2 by chromatography on a Sephacryl S-300 column. After reduction of F(ab')2 to Fab' by 2-mercaptoethylamine, the Fab' was labeled with peroxidase using N-succinimidyl 6-maleimidohexanoate.

ELISA procedures. ELISA was performed by a "Sandwich" method microtiterplater for ELISA was coated with 100 ul of 0.1 mg/ml anti-STS IgG in 50 mM NaHCO3 buffer (pH 9.6) overnight at 4°C. The plate was washed 3 times with phosphate buffered-saline (PBS) blocked with 2% (W/V) skim milk for 1 hour at room temperature. After washing 3 times with PBS containing 0.05% Tween20 (washing buffer), 100 μl of serum or a solution containing STS in phosphate-buffered saline was added to the wells and incubated at room temperature for 3 hour. After the plate was washed 4 times with the washing buffer, 100 μl of the peroxidase-conjugated anti-STS Fab' diluted at 1:100 was added and left for 3 hour at room temperature, followed by washing 5 times with the washing buffer. Two wells were added 100 μl of peroxidase substrate solution (3 mg of ophenylenediamine in 5 ml of 10 mM citrate phosphate, pH 5.0, containing 1.7 μl of hydrogen peroxide), and the reaction was allowed to develop at room temperature for 10 min. The reaction was stopped by adding 100 μl of 2 N H_2SO_4 and the absorbance was measured at 490 nm.

Results

Establishment of ELISA Method of Serum Steroid Sulfatase. STS was purified 120-fold with a yield of 6% from placenta. A single protein band was given indicating that STS has been purified to an apparent homogeneity from human placenta. Molecular weight was estimated approximately 63,000 on reducing SDS-PAGE (Fig.1). The extent of specific activity of the purified enzyme, 1,900 U/mg protein, was equal to that obtained previously[8].

Specificity of antibody against STS. To evaluate the specificity of the raised antibody, the STS preparation was subjected to SDS-PAGE and then transferred to a nitrocellulose sheet followed by immunoblot with the antibody. As shown in Fig. 2, the

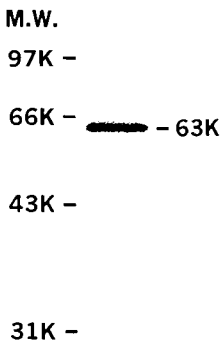


Fig. 1 SDS-PAGE of the purified STS from human placenta. The enzyme protein, 2ug was electrophresed on 10% acrylamide gel containing SDS. The band was visualized by silver-staining.

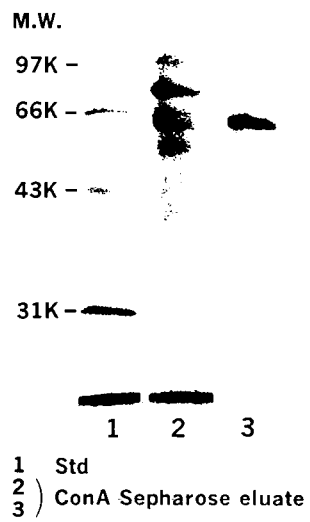


Fig. 2 Western blotting of a STS-containing fraction. The eluant on Con-A Sepharose was electrophoresed and immunobloted using anti-STS IgG. Lane 1, molecular standard markers; Lane 2, 3, eluant (about 40 μg proteins) from ConA Sepharose; Lane 1,2, with stained amido black; Lane 3, stained with peroxidase technique.

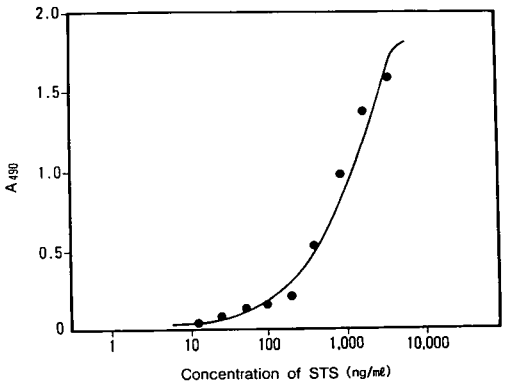


Fig. 3 Standard curve of the ELISA assay for STS enzyme protein.

antibody detected a single band at a position corresponding to molecular weight of the purified STS.

Sensitivity of ELISA. The standard curve using by this assay method as shown in Fig. 3, a range of 10 to 1500 ng/ml, STS protein was possible to assay.

Serum STS level in normal women and cancer patients. Normal level : The serum level of STS protein in healthy women (37.0 ± 13.6 year at the year of age, n = 69) was 74.5 ± 27.7 ng/ml (mean ± S.D.). On the other hand, the serum level of STS (43.0 ± 19.3 ng/ml) in normal men (33.7 ± 6.7 year at the year of age, n = 8) was lower than that in women.

Serum steroid sulfatase Gynecologic patients. Although serum STS concentrations of gynecologic patients were distributed somewhat in a wider range, the level in gynecologic cancer patients were elevated compared to that in healthy women (Fig. 4) The serum STS protein in

cervical carcinoma patients (117.8 ± 14.5 ng/ml) was significantly ($p < 0.05$) elevated than that in normal women. The serum STS in endometrial carcinoma patients (190.8 ± 31.3 ng/ml) was significantly ($p > 0.01$) higher than normal. The STS level (176.1 ± 22.6 ng/ml) was also significantly ($p < 0.01$) elevated in ovarian carcinoma. Cut-off value was determined at 130 ng/ml, which is Mean ± 2 SD of normal control.

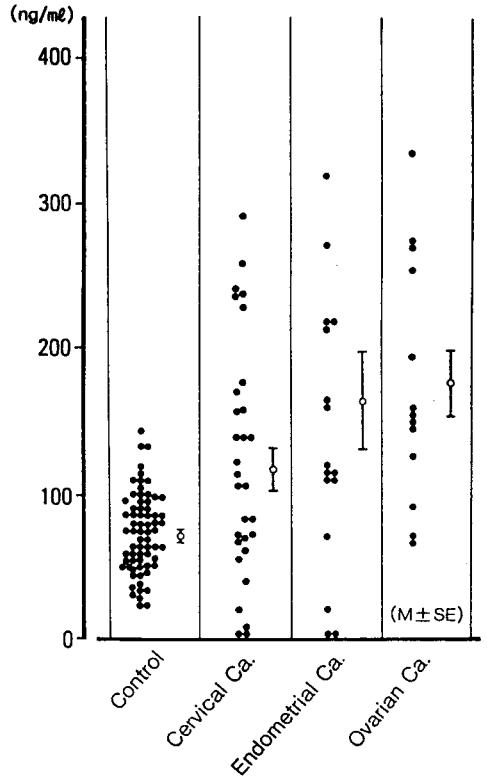


Fig. 4 The serum levels of STS in patients with cervical carcinoma, endometrial carcinoma, ovarian carcinoma. The cut-off value was set at the mean +2S.D.(130 ng/ml) of healthy female controls.

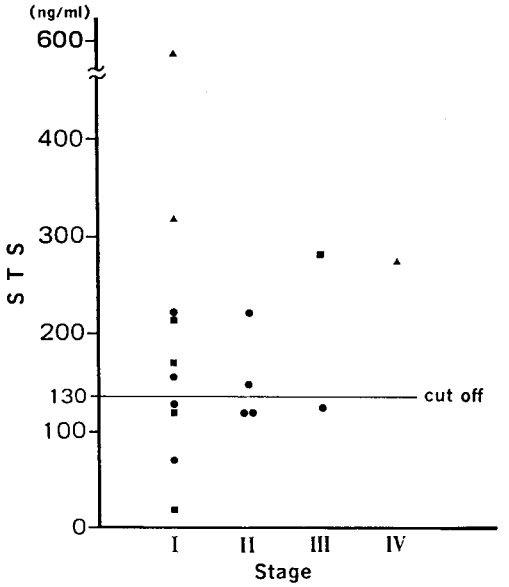


Fig. 5 Relationship between serum STS and staging in endometrial carcinoma. The histologic type was diagnosed as G1 (●), G2 (■) and G3 (▲). G1 ; highly differentiated adenomatous carcinoma. G2; moderated differentiated adenomatous carcinomas with partly solid areas. G3; predominantly solid or entirely undifferentiated carcinoma.

Relationship between serum steroid sulfatase and stage of cancer. The relationship between the serum steroid sulfatase, clinical stage and histology in patients with endometrial carcinoma and ovarian cancer are shown in Fig. 6. On the whole, stage dependent the serum steroid sulfatase levels could be observed. The serum STS of patients with histological grade 3 was higher than those of grade 1 and 2, independently clinical staging. Even though at stage, the elevation of that in patients with mucinous adenocarcinoma was noticeable. The serum levels of steroid

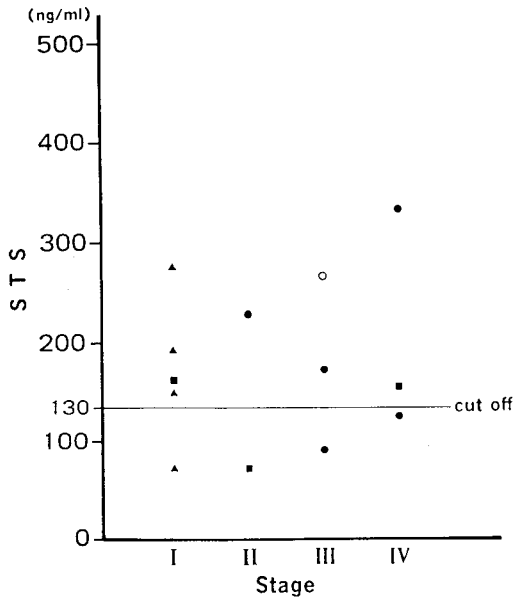


Fig. 6 Relationship between serum STS and staging in ovarian carcinoma. Each histological type was diagnosed as serous cystadenocarcinoma (●), mucinous cystadenocarcinoma (▲), clear cell carcinoma (O) and undifferentiated carcinoma (■).

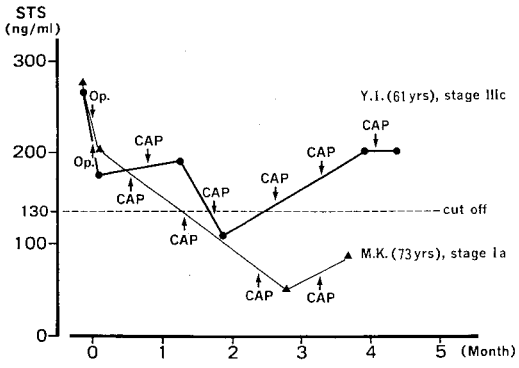


Fig. 7 Changes in serum STS levels in patients with ovarian carcinoma.

sulfatase in the patients of cervical squamous carcinoma did not have any relationship between the clinical stage (data not shown).

Effect of therapy on the appearance of steroid sulfatase. We examined for correlation between serum STS and clinical course in two patients with ovarian carcinoma. After surgery, the serum STS level decreased in patient with stage IIIc clear cell carcinoma, however, the lowest value was higher than the cut-off value. In these patients, the carcinoma did not respond to CAP (cyclophohosphamide, adriamycin and cisplatin) therapy, and that serum STS became elevated. On the other hand, the serum STS level of stage Ia with mucinous adenocarcinoma decreased as the tumor responded to the chemotherapy.

Discussion

Commonly, the enzymatic activity of STS is assayed using titrated steroid sulfate as a substrate. Such methodology precludes measuring the enzyme activity in serum where concentration of endogenous steroid sulfates is approximately 100 times higher than that of the exogenously added substrate[13]. To overcome this limitation we developed an ELISA method to measure the level of STS protein in serum. The present assay method enabled to assay STS protein as low as 10 ng/ml. In this method endogenous sulfated steroids which interfered the activity assay did not inhibit the assay.

The STS gene is localized on the X chromosome, and escapes from

Lyon's inactivation[14]. It may be for this process that the serum level of STS in normal men was lower than that in women.

Estrogen plays a role in promoting the development of endometrial cancers[4]. The endometrial carcinoma is associated with local production of estrone through aromatization of androstenedione[15,16] and desulfating estrone sulfate[7]. In post menopausal women, estrone sulfate is the most important estrogen precursor[17], and is concentrated higher than in pre menopausal women[18]. The steroid sulfatase activity in endometrial cancer tissue is significantly higher than in corresponding normal tissue[7]. It is probable that elevated STS enzyme activity in endometrial cancer tissue is reflected the increased level of STS protein serum. Normal ovary tissue has also STS activity[1] together with estrogen receptor. The estrogen receptor is present in some ovarian cancers, and the tumor tissues are responsive to estrogen by STS[5]. It is possible that the desulfatation of estrone sulfate may serve a role in the maintenance and promoting development of such ovarian tumors[6]. The elevation of serum STS level of cervical carcinoma was lower compared to that in endometrial carcinoma and ovarian carcinoma. These results suggest that STS may play a role in development and proliferation of gynecologic adenocarcinoma.

Although the releasing mechanism of STS, which is an integral endoplasmic reticulum protein, into blood circulation is unclear, it is postulated that STS leaks from destroyed cancer cells or is secreted from cancer cells.

Determination of serum STS protein by this newly developed ELISA will be useful for clinical application as a possible tumor marker in gynecologic carcinomas, especially of adenocarcinoma type.

References

1. Warren JC, French AP. Distribution of steroid sulfatase in human tissues. J Clin Endocrinol Metab 1965;25:278-282.
2. Payne AH, Lawrence CC, Foster DL, Jaffe RB. Intranuclear binding of 17 - estradiol and estrone in female ovine pituitaries following incubation with estrone sulfate. J Biol Chem 1973; 248:1598-1602.
3. Adessi GL, Nhuan TQ, Vingler P. In vivo and in vitro metabolism of estrone and estradiol-17B and their 3-sulfates in pregnant female guinea-pigs : a plausible prehormone role of estrogen-sulfates the maternal uterus. J Steroid Biochem 1982;16:107-116.
4. Hammond CB, Jelovsek FR, Lee KL, Creasman WT, Parker RT. Effects of long-term estrogen replacement therapy. Am J Obstet Gynecol 1979;133:537-547.
5. Nash JD, Ozols RF, Smyth JF, Hamilton TC. Estrogen and anti-estrogen effects on the growth of human epithelial ovarian cancer in vitro. Obstet Gynecol 1989; 73:1009-1016.
6. Milewich L, Porter J. In situ steroid sulfatase activity in human epithelial carcinoma cells of vaginal, ovarian, and endometrial origin. J Clin Endocrinol Metab 1987;65:164-169.
7. Urabe M, Yamamoto T, Naitoh K, Honjo

H, Okada H. Estrone sulfatase activity in normal and neoplastic endometrial tissues of human uterus. Asia-Oceania J obstet Gynecol 1989;15:101-106.

8. Yen PH, Allen E, Marsh B, Mohandas T, Wang N, Taggart RT, Shapiro LJ. Cloning and expression of steroid sulfatase cDNA and the frequent occurrence of deletions in STS deficiency: implication for X-Y interchange. Cell 1987;49:443-454.
9. Shapiro LJ, Cousins L, Fluharty A, Stevens RL, Kihara H. Steroid sulfatase deficiency. Pediat Res 1977;11:894-897.
10. Laemmi U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227: 680-685.
11. Levy H.B. and Sober H.A. A simple chromatographic method for preparation of gamma globulin. Proc.Soc.Exp. Biol.Med. 1960;103:250-254.
12. Yoshitake S., Imagawa M., Ishikawa E., Niithu Y., Urushiazki., Nishiura M., Kanazawa R., Kuroaski H., Tachibana S., Nakazawa N and Ogawa H. Mild and efficient conjugation of rabbit Fab' and horseradish peroxidase using a maleimide compound and its use for enzyme immunoassay. J. Biochem. 1982;92:1413-1424.
13. Loriaux DL, Ruder HJ, Lipsett MB. The measurement of estrone sulfate in plasma. Steroids 1971;18:463-472.
14. Muller CR, Migl B, Traupe H, Ropers HH. X-linked steroid sulfatase: Evidence for difference gene-dosage in males and females. Hum Genet 1980;54:197-199.
15. MacDonald PC, Siiteri PK. The relationship between the extraglandular production of estrone and the occurrence of endometrial neoplasia. Gynecologic Oncology 1974;2:259-263.
16. Yamaki J, Yamamoto T, Okada H. Aromatization of androstenedione by normal and neoplastic endometrium of the uterus. J Steroid Biochem 1985;22: 63-66.
17. Roberts KD, Rochefort JG, Bleau G, Chapdelaine A. Plasma estrone sulfate levels in postmenopausa women. Steroid 1980;35:179-187.
18. Jasonni VM, Bulletti C, Franceschetti F, Bonavia M, Bolleti G, Ciotti P, Flamigni C. Estrone sulfate plasma levels in postmenopausal women with and without endometrial cancer. Cancer 1984;53: 2698-2700.

Histologic Types of Ovarian Tumors in Maharaj Nakorn Chiang Mai Hospital

Surapan Khunamornpong MD,
Sumalee Siriaunkgul MD.

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

Abstract : *Based on histopathologic study, the relative frequency of ovarian tumors in Maharaj Nakorn Chiang Mai Hospital from January to December 1993 was analysed. Among 221 cases, 212 were primary neoplasms. Mature cystic teratoma was most commonly encountered (34%), followed by mucinous cystadenoma (19%) and serous cystadenoma (10%). For each histogenetic group, 51% of all primary tumors were common epithelial tumors, 7% were sex cord-stromal tumors, and 41% were germ cell tumors. In contrast to reports from western countries, mucinous tumor was more common than serous type with a ratio of 1.9:1 for benign group and 14:1 for borderline group. The relative frequency of granulosa cell tumor and malignant germ cell tumor was also higher than that of western countries. (Thai J Obstet Gynaecol 1995;7: 41-49.)*

Key words: ovary, tumor, histology

Histological typing of ovarian tumors, which was proposed by the World Health Organization (WHO) in 1973[1], has provided an internationally acceptable classification that can be used to investigate epidemiology and compare therapeutic results from different institution. Most of the published data, however, were from western studies[2-6] and only a small numbers were carried out in Asian countries including Thailand[7-9].

The purpose of this study was to analyze the frequency of various histologic types of ovarian tumors in Maharaj Nakorn Chiang Mai Hospital and to compare our findings with those presented in the International Union Against Cancer (UICC) Technical Report Series Volume 75[2].

Materials and Methods

The Maharaj Nakorn Chiang Mai Hospital (1500 beds) is the sole university hospital in Northern Thailand. The hospital serves not only as a primary health care center for

people in Chiang Mai province but also a referral center for other regional and community hospitals in the Northern region.

The cases included in this study were all ovarian tumors diagnosed in the department of Pathology between January and December 1993. Almost all cases (97.3%) had an adequate tissue sampling defined as minimum of 4 sections for benign tumors and 1 section per 1 cm. of the greatest dimension of the tumor particularly solid or papillary area for borderline and malignant tumors.

Hematoxylin and eosin stained (H&E) sections were performed routinely in each case. While special stains would be provided if necessary either histochemical stains (Mayers's mucicarmine, periodic acid Schiff and Grimelius) or immunohistochemical studies (antibodies for cytokeratin, carcinoembryonic antigen, alphafetoprotein, estrogen and progesterone related receptor protein, chromogranin, serotonin and myoglobin).

Each tumor was classified according to the standard nomenclature for ovarian tumors by the WHO classification. Ovarian endometriosis, simple cyst and tumor like lesions were excluded. Among the epithelial group, the borderline tumor (BT) or tumor of low malignant potential (LMP) was defined using the following criterion, presence of unusual proliferative activities characterized by nuclear atypia with stratification, increased mitotic activity, and tendency of separation of cellular buds floating in the lumen or on the surface. Presence of stromal invasion distinguished the malignancy or carcinoma from the BT or LMP. Another criteria for diagnosis of mucinous carcinoma, despite no evidence of stromal invasion, was nuclear atypia with stratification for 4 or more layers. Mixed epithelial tumor was diagnosed if more than one histologic type was seen in the same specimen and the minor element accounted for at least 10% of the representative tissue.

Additional information including age, laterality, and size of tumor were obtained from the pathology reports. The result of this study was compared to the comparative data of ovarian neoplasms from different geographic areas reported by Stalsberg et al[2]. To compensate the difference in age structure of the studies population, age standardized relative frequencies using the same unweighted mean percentage as Stalsberg's report were applied for statistical analysis.

Results

A total of 221 cases of ovarian neoplasms was histologically diagnosed during a one year period, of which 212 were primary and 9 were metastatic tumors. Table 1 listed the relative frequency of primary ovarian neoplasms. The most common tumor was mature cystic teratoma (73 cases or 34.4%). The next three most common tumors were mucinous cystade-

noma (41 cases or 19.3%), serous cystadenoma (21 cases or 9.9%) and mucinous LMP (14 cases or 6.6%). Among the 47 truly malignant tumors, endometrioid carcinoma (9 cases or 19%) was most commonly seen followed by serous carcinoma (8 cases or 17%) and granulosa cell tumor (6 cases or 12.8%).

Benign, LMP Malignant Tumors

The relative frequency of benign, LMP and malignant tumors is shown in Table 2. Benign tumors (149 cases) accounted for 70.3% while the LMP (16 cases) and malignant (47 cases) were 7.5% and 22.2% respectively. The total epithelial tumors (including 16 LMP) were 108 cases (50.9%), whereas, the sex cord stromal tumors were 15 cases (7%) and germ cell tumors were 88 cases (41.5%). Among the tumors classified as ovarian cancer (including LMP), 68% were epithelial type, 9.5% were sex cord stromal tumors and 20.6% were malignant germ cell tumors. If only truly malignant tumors were considered, epithelial tumors (27 cases) accounted for 57.4%. Sex cord stromal tumors (6 cases) and germ cell tumors (13 cases) shared 12.8% and 27.7% respectively.

Sixteen epithelial tumors were classified as LMP, of which 14 were intestinal mucinous LMP. The remaining two were serous and endometrioid type.

Table 1 *Relative frequency of primary ovarian neoplasms*

Primary ovarian neoplasms	No. of cases	%
Mature cystic teratoma	73	34.4
Mucinous cystadenoma	41	19.3
Serous cystadenoma	21	9.9
Mucinous borderline tumor (MLMP)	14	6.6
Endometrioid carcinoma	9	4.2
Fibroma/thecoma	9	4.2
Serous carcinoma	8	3.7
Gramulosa cell tumor	6	2.8
Clear cell carcinoma	4	1.9
Immature teratoma	4	1.9
Dysgerminoma	4	1.9
Endodermal sinus tumor	4	1.9
Mucinous carcinoma	3	1.4
Struma ovarii	2	0.9
Brenner tumor, benign	2	0.9
Carcinosarcoma	2	0.9
Other	6	2.8
	212	100

Table 2 *Relative frequency of types of primary ovarian neoplasms*

Type	No. of cases	%
Benign	149	70.3
Epithelial	65	
Sex cord stromal	9	
Germ cell	75	
Borderline (LMP)	16	7.5
Malignant	47	22.2
Epithelial	27	
Sex cord stroma	6	
Germ cell	13	
Other	1	

Table 3 *Frequency of subtypes of serous and mucinous tumors*

Type	serous	%	mucinous	%	Ratio of serous/mucinous
Benign	21	70.0	41	67.8	0.5
Borderline	1	3.3	14	25.4	0.1
Malignant	8	26.7	3	6.8	2.0
Total	30	100	58	100	

Comparison between serous and mucinous tumors was presented in Table 3. In the benign group, the number of mucinous tumor was almost twice that of serous tumor. This held true for LMP which the mucinous LMP was much more common than serous LMP (14:1). On contrary, the serous carcinoma was seen more frequently than mucinous carcinoma (8:3).

AGE

The frequency of primary ovarian tumors relating to the age distributions was shown in Table 4. It is notable that 73% of all benign tumors and 50% of all LMP occured in patients under the age of 45 years, while 33% of malignant epithelial tumors were seen in patients under 45 and only one of 27 cases (3.6%) was under 35. Malignant nonepithelial tumors were commonly encountered in patients under 45 (15 of 20 cases or 75%) and 76.9% of malignant germ cell tumors (10 of 13 cases) were seen in women younger than 35.

Table 4 *Types of tumors related to patient age*

Age	Benign	Borderline	Malignant		
			Epit.	Non-Epit.	Total
< 15	1	0	0	4	4
15-24	21	1	0	4	4
25-34	42	3	1	3	4
35-44	42	4	8	4	12
45-54	19	6	4	4	8
55-64	11	2	9	1	10
65-74	11	0	5	0	5
>74	1	0	0	0	0
unkown	1	-	-	-	-
Total	145	16	27	20	47

Bilaterality

Bilaterality of the tumors was found in 68.5% of serous carcinoma, 55% of endometrioid carcinoma, and 33% of mucinous carcinoma. Among the benign group, 13.7% of mature cystic teratoma as well as one Brenner tumor and one mucinous cystadenoma showed bilateral involvement. None of the serous cystadenoma had bilateral tumors.

Metastatic Tumor

Of all 9 metastatic tumors to the ovary, 7 were adenocarcinoma, one was squamous cell carcinoma, and one was high grade stromal sarcoma.

Comparative Data

Based on the same unweighted mean percentage as Stalsberg's report, the age standardized relative frequencies were calculated and compared to data from different geographic areas including Sendai (Japan), Bombay (India), Recife (Brazil), New Orleans (USA), Birmingham (England) and Norway.

Figure 1 shows the proportion of benign, LMP and malignant epithelial neoplasms. Range of proportion from 2% (Bombay and N.O. black) to 16.3% (Chiang Mai) was observed in the LMP. Proportion

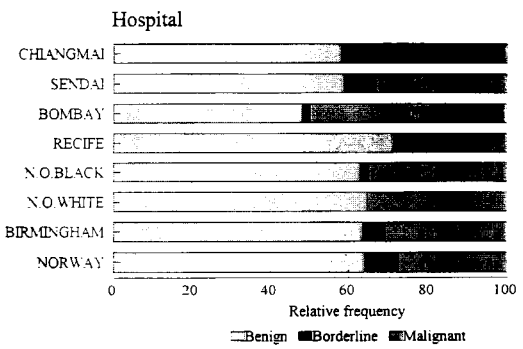


Fig. 1 Relative frequency of subtypes of epithelial tumors

of the two most common benign epithelial tumors, serous and mucinous, was presented in Figure 2. Serous cystadenoma was more frequently encountered than mucinous tumors in New Orleans (55:26.7 and 64:36) and Birmingham (54.8:43.6). In Sendai (43.8:49.7) and Chiang Mai (30.5:64.9), however, the serous type was less common than the mucinous. The highest proportion of serous cystadenoma was reported from Recife, but it was noted that non-neoplastic tumor like lesions were included in the same category.

Figure 3 shows the ratio of serous to mucinous tumor among benign, LMP and malignant subtypes. The lowest ratio for the LMP was 0.1 (Chiang Mai). The ratio was also lower in Sendai and Bombay than in Birmingham and Norway. These findings indicate that mucinous LMP was more common in Asian countries.

For the sex cord stromal tumors, the proportion of granulosa cell tumor and thecoma fibroma was presented in Figure 4. Higher proportion of the granulosa cell tumors (37.1 to 40.5%) was found in developing countries and African American while a lower frequency (5.4 to 20.4%) was seen in white and Japan (Sendai). Finally, Figure 5 indicate that the proportion

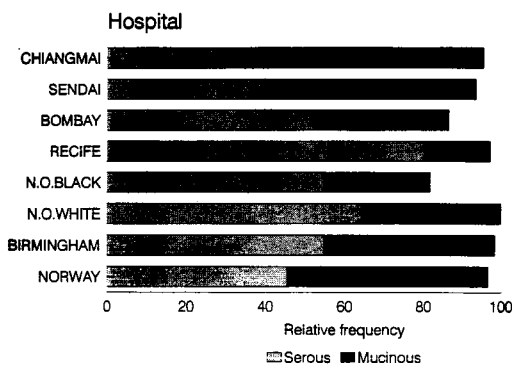


Fig. 2 Relative frequency of benign common epithelial tumors

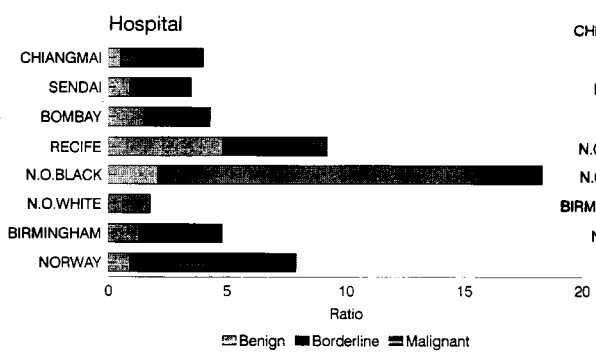


Fig. 3 Ratio of serous to mucinous tumors

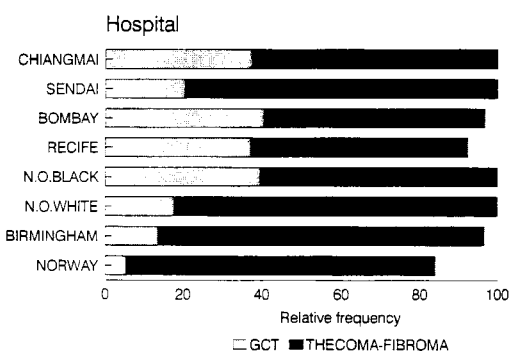


Fig. 4 Relative frequency of sex cord-stromal tumors

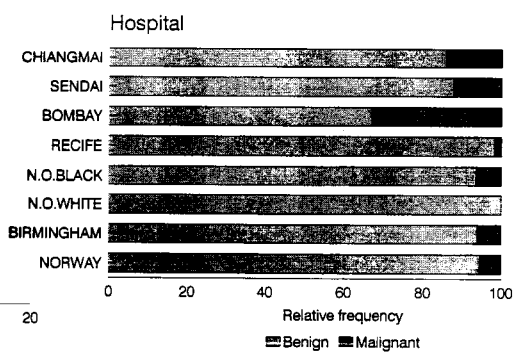


Fig. 5 Relative frequency of germ cell tumors

of malignant germ cell tumor was higher in Asian countries (12.5 to 33.0%) than that in western countries (0 to 6.3%).

Discussion

An international comparison of histologic types of benign and malignant ovarian tumors in general hospital material reported by Stalsberg et al. has provided the relative frequencies of each histogenetic group that 54% of all ovarian neoplasms were common epithelial tumors, 10% were sex cord stromal tumors and 35% were germ cell tumors[2]. In our study, the common epithelial tumors accounted for 50.9% with 7% of sex cord stromal tumors and 41.5% of germ cell tumors. Although these proportions seemed to be comparable, remarkable dissimilar findings in five subtypes were also noted and each will be discussed separately.

For the benign epithelial tumor, western countries reported that the serous cystadenoma was more common than mucinous cystadenoma with a ratio ranging from 1.3:1 to 2.2:1[2-4]. Our study and another report from a university hospital in Bangkok (Thailand)[9] showed that the serous type was less common than the mucinous type with a ratio of 0.5:1 and 0.4:1 respectively. This histologic distribution may be influenced by geographic difference rather than the use of variable diagnostic criteria.

Within the group of tumors of low malignant potential (LMP), a higher proportion of the mucinous LMP than the serous LMP in our study was supported by another two reports from the university hospital in Bangkok (Thailand)[8-9] and a report from Japan[7]. The ratio of serous to mucinous LMP from these studies ranged from 0.1 to 0.5, whereas, such a ratio from the western countries ranged 1.4 to 2[3-4]. Although the different distribution may possibly be due to varied diagnostic criterion to distinguish between benign and LMP, and between LMP and malignant tumors, the geographic influence also, perhaps, played a significant role.

As shown in Figure 1, the proportion of LMP varied from area to area and ranged 2.0 to 16.3% of all epithelial neoplasms. The difference may be influenced partly by different distribution in each area and partly by variation in the diagnostic criteria used among each institution.

Diagnosis of LMP was a subject of variability among pathologists. Although diagnostic criteria to differentiate LMP from either benign or malignant type has already been described[10], diagnostic decision was mainly depended on an individual subjectivity which was affected by variation of threshold from person to person. This problem was a well-recognized cause of under report of LMP and less reliability of incidence LMP in the cancer registries. Some LMP may be diagnosed as benign tumor, whereas some may be diagnosed as malignant. In our study,

6 of 16 LMP were in-itially reported as benign tumors.

The proportion of the truly malignant epithelial tumors in our study was also different from western studies. In the present report, carcinoma of the ovary accounted for 57.4% of all malignant tumors. This percentage was comparable to the report from Japan (63.9%)[7] but lower than that of reports from Western countries (85.5 to 92.3%)[3-4]. In contrast, the malignant germ cell tumors were more commonly seen in Asian countries, accounted for 20.6% of ovarian cancer in our study and ranged from 17.2 to 25% in the report from Japan[7]. This proportion was only 2.5 to 7% in the Western series[3,4,6]. We agreed with Nakashima et al. that higher relative frequency of the malignant germ cell tumors was considered to reflect a much lower relative frequency of malignant epithelial tumors. Comparison between various histologic subtypes of both malignant epithelial and germ cell tumors was limited because our study had smaller numbers of malignant tumors.

Among the sex cord stromal group, the proportion of the granulosa cell tumor in our study (37.6%) as well as in other developing countries (40.5% in Bombay, 37.1% in Recife) and African American (39.6%) was higher than that of reports from Japan and other developed areas (5.4 - 20.4%)[2,3].

In conclusion, the present study report the histologic distribution of ovarian tumors during a one year period in Maharaj Nakorn Chiang Mai Hospital. Comparison between our data and other reports especially from western countries showed remarkable different findings including 1) in this study, the mucinous cystadenoma and mucinous LMP were more common than the serous type 2) the relative frequency of the malignant epithelial tumor was lower, whereas, that of the malignant germ cell tumor was higher than in western countries 3) the relative frequency of granulosa cell tumor was higher in developing countries and African American.

References

1. Serov SF, Scully RE, Sobin LH. Histological Typing of Ovarian Tumors. International Histological Classification of Tumors. World Health Organization; 1973: No 9.
2. Stalsberg H, de Carvalho ARL, Correa P, et al. International comparisons of histologic types of benign and malignant ovarian tumors in general hospital material. In Stalsberg H, ed. An international survey of distributions of histologic types of tumors of the testis and ovary. UICC Technical Report Series: 1983; vol 75:313-330.
3. Katsube Y, Berg JW, Silverberg SG. Epidemiologic pathology of ovarian tumors: A histopathologic review of primary ovarian neoplasms diagnosed in the Denver Standard Metropolitan Statistical area, 1 July - 31 December 1969 and 1 July - 31 December 1979. Int J Gynecol Pathol 1982; 1: 3-16.
4. Koonings PP, Campbell K, Mishell DR, et al. Relative frequency of primary

ovarian neoplasms: A 10-year review. Obstet Gynecol 1989;74:921-926.

5. Hartge P, Schiffman MH, Hoover R, et al. A case control study of epithelial ovarian cancer. Am J Obstet Gynecol 1989;161:10-16.
6. Yancik R. Ovarian cancer. Age contrasts in incidence, histology, disease stage at diagnosis and mortality. Cancer (supp) 1993;71:517-523.
7. Nakashima N, Nagasaka T, Fukata S, et al. Study of ovarian tumors treated at Nagoya University Hospital, 1965-1988. Gynecol Oncol 1990; 37 :103-111.
8. Niruthisard S. Common epithelial cancers of the ovary at Chulalongkorn Hospital (1985 - 1989). Chula Med J 1991; 35: 735-743.
9. Isarangkul W. Ovarian epithelial tumors in Thai women: a histological analysis of 291 cases. Gynecol Oncol 1984;17:326-339.
10. Scully RE, Salazer H. Problems in the histological typing of ovarian type specific cancer incidence rates in several countries. In Stalsberg H, ed. An international survey of distributions of histologic types of tumors of the testis and ovary. UICC Technical Report Series: 1983; vol 75:123-136.

Platinum Based Chemotherapy for Advanced Epithelial Ovarian Cancer (AEOC)

Saibua B. Chichareon MD,
Surat Wattanakitkrailert MD.

Gynecologic Oncology Unit, Department of Obstetrics and Gynecology,
Faculty of Medicine, Prince of Songkla University, Hat-Yai, Songkhla, Thailand.

Abstract : *Between January 1987 to December 1989, thirty-seven patients with advanced epithelial ovarian cancer were treated with various Platinum based chemotherapy according to their financial status. Regimen A consisted of cisplatin, doxorubicin and cyclophosphamide. Regimen B was cisplatin and melphalan. Overall response rates were 80% and 86% for regimen A and B, respectively and median duration of response were 21 and 15 months. The median survival was more than 35 months in both regimens. The proportions of "non responders" was similar in both treatment groups and the proportions in remission at various times following onset of treatment were also similar. When considering the cost effectiveness of treatment of 100 cases of advanced ovarian cancer, the reduction in costs of $ 77,160 would be obtained at the expense of 13 cases failing to survive past 35 months. (Thai J Obstet Gynaecol 1995;7:51-60.)*

Key words : cost effectiveness, advanced epithelial ovarian cancer

Cancer management causes a heavy financial burden to a goverment referral center. Songklanagarind Hospital is a university hospital with complete facilities for treatment of gynecologic cancer. In cases of advanced epithelial ovarian cancer, adjunctive chemotherapy is necessary. Available commercial chemotherapeutic drugs are imported and costly for a developing country like Thailand. Because most of the patients are poor, and have no health insurance, the burden falls largely on the hospital to provide comprehensive treatment. To do so, under the constraint of limited resources, two different postoperative chemotherapy protocols for advanced epithelial ovarian cancer were used. The first was a regimen of cisplatin, doxorubicin and cyclophosphamide (regimen A) and prescribed for affordable patients. The other, regimen B, was the combination of cisplatin and melphalan (phenylanine mustard, L-PAM) and used for indi-

gent patients. This regimen shared comparable activity against ovarian cancer[1-5]. The objective of this study was to compare the results and cost effectiveness of the two treatments.

Materials and methods

From January 1987 to December 1989, patients with advanced epithelial ovarian cancer (stage III or IV, International Federation of Gynecology and Obstetrics classifications) were studied. Histologic confirmation of the disease and the end of disease was required. The end of the study was on 30 April, 1991.

The primary surgery was performed at the Department or elsewhere. In cases where the operation was initially performed at another hospital, a surgical summary indicating the extent of the disease at laparotomy was sought through personal or official contact.

Patients were categorized according to their financial status. Those affordable patients received regimen A. Those indigent patients received regimen B. The chemotherapeutic drugs were started 2-4 weeks after surgery unless delayed by postoperative complications.

The drug doses in regimen A were intravenous 50 mg/m^2 of cisplatin, 50 mg/m^2 of doxorubicin and 200 mg/m^2 of cyclophosphamide on the same day. Prehydration was given before cisplatin infusion with one half normal saline intravenously at a rate of 300-500 ml. per hour for 2-4 hours and 100 ml. 20% mannitol infused in 20 minutes. Another 1000 ml. of one half normal saline was also infused intravenously in 6 hours immediately after cisplatin infusion. The dose of cisplatin and technique of administration in regimen B were the same as in regimen A followed by melphalan given orally 0.2 mg/kg for five days.

Courses of both regimens were repeated every 28 days. provided there was no evidence of toxicity. Six courses of therapy were planned. Patients who received at least 3 courses of therapy were included in the analysis. Although the clinical evaluation which consisted of physical and pelvic examination were performed before the beginning of the next course, the evaluation after receiving three courses of chemotherapy was the first documented response. After 6 courses of therapy, if the patients were clinically free of disease, they would undergo a reevaluation with Chest X-ray and abdominopelvic ultrasonographic studies. If the result of the studies yielded negative findings, a second look would be offered. Patients who refused a second look laparotomy were considered for a 3-6 courses of additional chemotherapy. The schedule of followup was every 4 and 8 weeks in the first and second year, respectively.

Complete response was defined as the disappearance of all evidence of disease as judged by clinical examination and investigation, in particular,

Partial response was defined as more than 50% reduction in the size of the measurable tumor. Duration of response was recorded as the time between the earliest examination at which a response had occurred, and the time at which a reappearance or metastasis of tumor was detected. Patients showing no response (stable or progressive disease) were recorded as having a response duration of zero. During the study period, if the patients received postoperative chemotherapy but they failed to attend follow up programme or never response the questionnaire sent by mail, they were signed as lost to follow up. Patients who were lost to follow up, who had not yet developed recurrence by the end of the study, were considered to be censored. Response curves were constructed by Kaplan and Meier's method[6].

Survival duration was recorded from the date of treatment which was the date of the first operation. Patients who were lost to follow up, or who were still alive at the end of the study, were considered to be censored. Both response and survival curves were constructed by the methods of Kaplan and Meier[6].

Response and survival profiles of the two treatment groups were compared using Chi-square statistic, Fisher-exact test and Log-rank test. Confidence intervals for response and survival propabilities at specified durations were calculated from the values of standard error determined by the method of Peto[7].

For cost effectiveness analysis, only direct costs were identified. Indirect and intangible costs were excluded as it was difficult to allocate a monetary value to these. Direct costs comprise of direct medical costs and non medical direct costs. The former were the cost of hospitalization, drugs, laboratory tests, investigation procedures, pathological examination, and operation fee. The latter were food, transportation, and lodging. It was emphasied that the cost of treatment was calculated under the assumption that no medical or surgical complication occurred and the patients received 6 courses of chemotherapy. The effect in this situation was the number of patients surviving 3 years after treatment.

Results

During the 3 year period, there were 96 patients with epithelial ovarian cancer. Fifty six patients had advanced stages. Of these, 37 were evaluable in that they had received at least 3 courses of the chemotherapy. Patients excluded were those who refused therapy or received less than 3 courses.

The patient characteristics were shown in Table 1. All except one in regimen B were in stage III. The initial operation in more than 60% of patients were total abdominal hysterectomy with bilateral salpingo-oophrorectomy plus omentectomy. The proportion of optimal cytoreduction as well as the post perative

complications in both groups were not different. The common histology of tumor was serous type. Although a second look operation was planned for clinically complete responders, most of the patients refused. Only 3 cases (2 in regimen A and 1 in regimen B) underwent a second look operation and revealed no macroscopic disease. The mean courses of chemotherapy was 7 and 8 in regimen A and B respectively. Although the patients in each group were different in financial status, the follow up times was similar in both regimens ranging from 4 to 39 months in regimen A and 3 to 35 months in regimen B. Median follow up time in both groups were approximately 14 months.

The over all response (complete and partial) were 80% and 86% for regimen A and B, respectively (Table 2.) Response curves and survival curves in the two treatment groups are shown in Figs. 1 and 2, respectively. Median response time were 21 months in regimen A and 15 months in regimen B (non responders included; Table 3.).

Toxicity was tolerable in both regimens. Table 4. listed three major toxic effects and their frequency. Hematological toxicity was more common in regimen B. Almost all patients developed alopecia, anorexia and vomiting. Severe vomiting was more common in regimen A. Electrocardiography of one patient in regimen A showed first degree heart block after receiving 5 courses of chemotherapy. Liver enzymes have definitely increased in 2 patients, of whom had liver scan for suspected liver metastasis but ultrasonography and laparotomy findings yielding negative result. Extravasation rendered skin necrosis occurred only once.

Details of the costs for patients receiving initial surgical treatment were shown in Table 5. The cost of initial surgery was the same in both regimens, $ 160. The costs per patient per course were $ 202 and $ 73.4 in regimen A and B respectively. The laboratory cost of regimen A was expensive than regimen B because of electrocardiography which had to be performed in order to monitor possible cardiotoxicity of doxorubicin. Under the assumption that a patient received surgical treatment plus 6 courses of chemotherapy without complications or serious side effects, the total cost was $ 1372 for regimen A and $ 600 for regimen B. The costs and effects of the treatment of 100 cases of advanced ovarian cancer using two different regimen are shown in Table 7. The effect was defined on the basis of number of patients surviving 35 months after treatment. The saving in cost comparing regimen B with A was $ 77,160, while the effect was 13 cases failing to survive 35 months.

Discussion

The main obstacle in management for advanced ovarian cancer in developing country, such as Thailand, are the high cost of treatment and low

Table 1 *Characteristics of Patient Groups*

Variable	Regimen A(CAP)	Regimen B (PM)
Total number assessed	15	22
Age (yr)		
Range	16-72	23-72
Median	48	53
FIGO stage (no. of patients)		
III	11	21
IV	4	1
Initial operation (no. of patients)		
Biopsy only	1	1
Excision of mass	2	2
TAH/BSO plus omentectomy	10	17
BSO plus omentectomy	1	1
Omentectomy	1	1
Histology of tumor		
Serous	8	10
Mucinous	3	5
Clear cell	1	2
Endometrioid	1	4
Mixed type	-	1
Mixed mullerian	1	-
Undifferentiated	1	-
Mean number of courses of chemotherapy	7	8
Follow up times (months)	4-39	3-35

Note. CAP, cisplatin/doxorubicin/cyclophosphamide; PM, cisplatin/melphalan; TAH/BSO, total abdominal hysterectomy/bilateral salpingo-oophrorectomy.

Table 2 *Results of Therapy*

Results	Regimen A (CAP) (n=15)	Regimen B (PM) (n=22)	ρ value (Fisher Exact)
No. responding:	12 (80%)	19 (86%)	0.67
Complete response	8 (53%)	13 (59%)	0.75
Partial response	4 (27%)	6 (27%)	
No response	3 (20%)	3 (14%)	

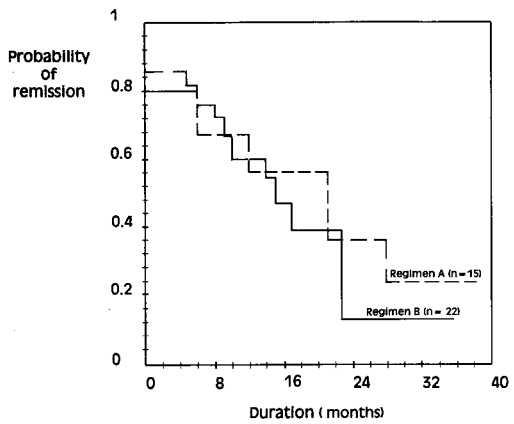


Fig. 1 **Remission curves in the two treatment groups of patients with advanced epithelial ovarian cancer.**
Regimen A = cis-platinum + doxorubicin + cyclophospha-mide.
Regimen B = cis-platinum + melphalan.

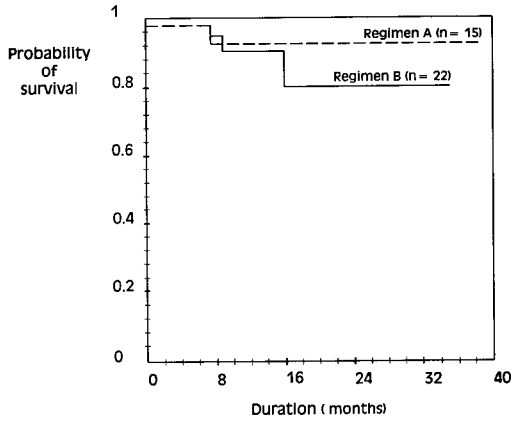


Fig. 2 **Survival curves in the two treatment groups of patients with advanced epithelial ovarian cancer.**
Regimen A = cis-platinum + doxorubicin + cyclophospha-mide.
Regimen B = cis-platinum + melphalan.

Table 3 *Comparison of Treatments*

Parameter	Regimen A (CAP) (n=15)		Regimen B (PM) (n=22)
No. censored	6		9
1-year response, %	57 (30,85)		61 (39, 82)
2-year response, %	34 (11, 57)		12 (0, 25)
35-month response, %	23 (0, 46)		12 (0, 25)
Median duration of response, months	21 (6, 21)		15 (9, 21)
Chi-square for homogeneity		0.152	
ρ value (log rank test)		0.70	
No. censored	14		19
1-year survival, %	92 (76, 100)		88 (72, 100)
2-year survival, %	92 (77, 100)		79 (57, 100)
35-month survival, %	92 (77, 100)		79 (57, 100)
Median survival time, months	>39		>35
Chi-square for homogeneity		0.385	
ρ value (log rank test)		0.53	

Note Non responders were included. Values in parenthesis were 95% confidence limits.

Table 4 *Toxicity from chemotherapy*

	Regimen A (CAP) (n = 15)	Regimen B (PM) (n = 22)
Leukopenia*	-	1
Thrombocytopenia**	1	5
Anemia**	-	7
Vomiting (severe)	5	2
Cardiotoxicity	1	-
Hepatotoxicity	-	2
Skin necrosis	1	-

*1000-2000 leukocytes/mm^3
**<100,000 platelet/mm^3
***hemoglobin<10gm/dL

Table 5 *Cost identification Components of cost with surgery in patient with epithelial ovarian cancer*

Direct costs	U.S $
Direct medical cost	
Hospitalization	9.4
Drugs	
Antibiotic	6.4
Other durgs	1.2
Intravenous line	10.6
Enema & vaginal douche	1.6
Cross match for blood transfusion	14.4
Laboratory test	14.5
X-ray procedure	3.6
Pathological examination	8.0
Operation fee	40.0
Anesthetic fee	28.0
Non-medical direct costs	
Food	10.8
Transportation	8.0
Lodging	1.8
Total	160.0

Note Indirect and intangible cost has been excluded.

Table 6 *Cost identification Component of costs per patient per course of adjuvant chemotherapy*

Direct costs	Regimen A(CAP) U.S. $	Regimen B (PM) U.S. $
Direct medical cost		
Hospitalization	2.1	2.1
Drugs		
Chemotherapy	166.5	41.5
Other drugs	0.8	0.8
Intravenous line	4.8	4.8
Laboratory test	14.6	11.0
X-ray procedures	3.6	3.6
Non-medical costs		
Food	1.2	1.2
Transportation	8.0	8.0
Lodging	0.4	0.4
Total	202.0	73.4

Note Indirect and intangible cost had been excluded.

Table 7 *Cost effectiveness analysis Costs and effects on survival of different regimen for 100 patients with advanced epithelial ovarian cancer*

Strategy	Cost U.S. $	Effects (no. of patients survival at 35 months)
Surgery + CAP	137,200	92
Surgery + PM	60,040	79
Increment cost and effects	77,160	13

Note Surgery plus 6 courses of chemotherapy without complications or serious side effects.

patient compliance. From the research viewpoint, the first obstacle renders a randomized clinical trial which is rather difficult to implement. The latter obstacle was reflected, in this study, by the high rate of refusal or discontinuation of therapy and patients lost to follow up.

Adjuvant chemotherapy in advanced ovarian cancer is necessary since it can improve the median survival time, although the long term survival is still disappointing.[8-11] The combination chemotherpy regimen should minimally consist of a platinum compound and an alkylating agent. The response rate in cisplatin based regimen is expected to be about 60%[12-15]. Although the median duration of response in the current study

are not dissimilar from those in other studies[15-18], the response rate of 80% and the more than 35 months median survival indicate a surprisingly good outcome. A high response rate, however, dose not guarantee a high survival rate. The 5 year survival in other studies is less than 30%.[8,9,11] This study was limited by the small number of patients, the non-randomized allocation to treatment regimens, the high proportion of censored data resulting in wide confidence intervals, and the relatively short follow up times. However, the data are consistent with a duration of partial or complete response to cisplatin plus melphalan similar to the standard regimen. The proportion of "non responders" was similar in both treatment groups, with the proportion in response at various times following onset of treatment.

The most common side effects of cisplatin were nausea and vomiting which occurred in all patients. Fortunately, it was tolerable. Hematological toxicity, including neutropenia and thrombocytopenia was common in patients who received melphalan as it causes a prolonged course interval time.

The total costs for treatment of advanced epithelial ovarian cancer using standard regimen was two times of the alternative regimen. For a cost saving of $ 77,160 per 100 patients, an additional 13 patients may fail to survive beyond 35 months. Evaluating reduced patient survival with savings in treatment cost was very difficult. However, under conditions of limited resources, providing expensive treatment to benefit a few may not be economical appealing. The saving in cost of $ 77,160 in some opinions may not be a large amount of money, but in a developing country whose mean annual income per capita is $ 1,170[19], it represents a relatively large sum. In the management of cancer patients, these aspects should be considered when selecting the treatment protocol. These include efficiency, toxcity, and cost effectiveness. The first is the efficiency, the second is an acceptable toxicity and the third is the cost effectiveness.

Acknowledgements

The authors wish to thank Dr. Alan Geater for his statistical advice.

References

1. Young RC. Chemotherapy of ovarian cancer: Past and present. Semin Oncol. 1975;2:267-276.
2. Young RC. Chabner BA, Hubbard SP, Fisher RI, Bender RA, Anderson T, Simon RM, Canellos GP, and DeVita VT. Advanced ovarian adenocarcinoma: A prospective clinical trial of melphalan (L-PAM) versus combination chemotherapy. N Engl J Med 1978;299:1261-1266.
3. Smith JP, and Rutledge FN. Chemotherapy in the treatment of cancer of the ovary. Am J Obstet Gynecol 1970;107: 691-703.
4. Wiltshaw E. A review of clinical experience with cis-platinum diamminedichloride. Biochimie 1978;60:925-929.

5. Sessa C. European studies with cisplatin and cisplatin analog in advanced ovarian cancer. Eur J Cancer clin Oncol 1986; 22:1271-1277.
6. Kaplan EL, and Meier P. Nonparametric estimation from incomplete observations. J Ann Stat Assoc 1958;53:457-481.
7. Peto R, Pike MC, and Armitage P. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. Br J Cancer 1976;35: 1-39.
8. Sutton GP, Stehman FB, Einhorn LH, Roth LM, Blessing JA, and Ehrlich CE. Ten year follow up of patients receiving cisplatin, doxorubicin, and cyclophosphamide chemotherapy for advanced epithelial ovarian carcinoma. J clin Oncol 1989;7:223-229.
9. Hainsworth JD, Grosh WW, Burnett LS, Jones HW, Wolf SN, and Greco FA. Advanced ovarian cancer; Long term results of treatment with intensive cisplatin based chemotherapy of brief duration. Ann Int Med 1988; 108:165-170.
10. Neijt JP, Van der Burg MEL, Van Oosteron AT. Combination chemotherapy in advanced ovarian cancer: The experiences of the Netherlands Joint study Group for Ovarian cancer. In: Conte PE, Ross R, Ragni N, Vermorken JB, eds. Multimodal treatment of ovarian cancer. New York: Raven Press, 1989:227-235.
11. Belinson JL, Lee KR, Jarrell MA, and McClure M. Management of epithelial ovarian neoplasms using a platinum-based regimen: A 10 year experience. Gynecol Oncol 1990;37:66-73
12. Weiss GR. Second-line chemotherapy for ovarian cancer. Clin Obstet Gynecol 1986;29:665-677.
13. Wiltshaw E, Evans B, and Harland S. Phase III randomised trial cisplatin versus Jm8 (carboplatin) in 112 ovarian cancer patients, stages III and IV. Proc Am Soc Clin Oncol 1985;4:121.
14. Adams M, Kerby IJ, Rocker I, Evans A Johansen K, and Feanks CR. A comparison of the toxity and efficacy of cisplatin (CDDP) and carboplatin (JM8) in advanced ovarian cancer. Proc Am Soc Clin Oncol 1987;4:207.
15. Conte PF, Bruzzone M, Sertoli MR, Daga MG, and Rubagotti A.A randomized trial comparing cisplatin plus cyclophosphamide versus cisplatin, doxorubicin, and cyclophosphamide in advanced ovarian cancer. J Clin Oncol 1986;4:965.
16. Decker DG, Fleming TR, and Edmonson JH. Cyclophosphamide plus cis-platinum in combination: Treatment program for stage III or IV ovarian carcinoma. Obstet Gynecol 1982;60:481-487.
17. Barker GH, and Wiltshaw E. Randomised trial comparing low-dose cisplatin and cholorambucil with low-dose cisplatin, chlorambucil, and doxorubicin in advanced ovarian carcinoma. Lancet 1981;4:747-750.
18. Gruppo Interregionale cooperativo Oncologic Ginecologia. Randomised comparison of cisplatin with cyclophosphamide/ cisplatin and with cyclophosphamide/ doxorubicin/ cisplatin in advanced ovarian cancer. Lancet 1987;15: 353-359.
19. World population data sheet, the Population Reference Bureau Inc., 1991.

Optimal Dosage of Carboplatin (CP) in Advanced Epithelial Ovarian Cancer (AEOC)

Sumrit Senapad Dr.med, FICS, FRTCOG,
Somchaya Neungton MD,
Chaiyod Teerapagawong MD, FRTCOG,
Veerasak Thaidhanisawan MD, FRTCOG.

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

Abstract : *The evaluation of efficacy and toxicity of combined carboplatin (CBP) in two different doses with fixed dose of cyclophosphamide (CTX) for treatment of advanced ovarian epithelial cancer was performed. The patients were treated by these combination after randomisation every 3 weeks for 8 cycles. Arm A (CBP 300 mg/m² + CTX 500 mg/m²) achieved the over all response rate of 70.0% and clinical complete response rate of 50.0%, arm B (CBP 400 mg/m² + CTX 500 mg/m²) had over all response and complete response rate of 80.0% and 75.0% respectively (p = 0.1). The pathological complete response showed in both arms 77.77% and 76.92% (P = 0.39), with recurrence 28.57% and 20.0% (P = 0.39). The survival rate were constructed by Kaplan and Meier's method, with 3 years survival rate of 40.0% and 55.0% respectively (P = 0.14). The median survival was 25.0 months in arm A, and 35.5 months in arm B (P = 0.29). The haematologic toxicity grade 4 was noted in few patients of both arms. Nausea and vomiting were minimal after prophylactic antiemetic. No hepatotoxicity, nephrotoxicity and neurotoxicity were noted. (Thai J Obstet Gynaecol 1995; 7:61-68.)*

Key words : advanced ovarian epithelial cancer, chemotherapy

Ovarian cancer is the most frequently diagnosed at an advanced stage. Only minority of patients have surgically curable disease, thus systemic chemotherapy has become the major treatment modality. The current platinum base regimens yield substantial pathologic complete response rate between 33.3-83.3%.[1-7] [34.4[1], 70.0[2], 50.0[3], 40.0[4], 33.3[5], 45.0[6] and 83.3 per cent[7]] The cisplatinum showed the nephrotoxic, neurotoxic, emetogenic side effects and required pretreatment hydration. Carboplatin is a second generation platinum compound that is less nephrotoxic,

neurotoxic and emetogenic than the cisplatinum. Thus carboplatin can be administered without hydration due to absence of nephrotoxicity.[8,9] The combined carboplatin and cyclophosphamide showed the clinical complete response 33.0%[10] The study was designed to evaluate the efficacy of combined carboplatin in two different doses with fixed dose of cyclophosphamide in treatment of advanced epithelial ovarian cancer post surgery. The observation included response rates, haematologic and non haematologic toxicities, and survival at least 3 years.

Patients and Methods

Patient selection : Eligibility criteria included : histological proof of epithelial ovarian cancer, stage III-IV disease, after total hysterectomy with bilateral salpingo-oophorectomy and infracolic omentectomy or partial resection of the tumour or only biopsy, age < 75 years, performance status < 3, adequate bone marrow function (a leukocyte count greater than 4,000/Cu.mm., a platelet count greater than 100,000/Cu.mm., haemoglobin level greater than 8 gm/100 ml.), adequate liver function (SGOT level less than 100 sigma unit), normal renal function (serum creatinine level less than 2 mg/100 ml.) and verbal or signed the consent form.

Randomisation : The randomisation was allocated by computer.

Treatment plan and doses modifications : Patients were randomised to receive either carboplatin 300 mg/m^2 + cyclophosphamide 500 mg/m^2 intravenously in day 1 (arm A), or carboplatin 400 mg/m^2 + cyclophosphamide 500 mg/m^2 intravenously in day 1 (arm B). The cyclophosphamidly was given in water 20 ml intravenous push slowly, and carboplatin was given in 5% dextrose 500 ml intravenously for 2 hours. The patient received haloperidol 10 mg intravenously before chemotherapy for prophylactic antiemetic. The treatment would be repeated every 3 weeks for 8 cycles, except in the patient who showed no response would be treated by second line drugs. Subsequent courses of treatment; if the total white count was less than 3000/cu.mm, platelets less than 100,000/cu.mm, or haemoglobin less than 8 gm/100 ml, the treatment was delayed for 1 week. If these values were not reached after 1 week, the supportive treatment was performed such as oral steroid and blood transfusion, the drugs was decreased 50% for the haematologic toxicity grade 3,4. The carboplatin dose was decreased 50%, if the SGOT level reached 2x normal or creatinine level > 2 mg/100 ml, but the cyclophosphamide was given in fixed dose.

The non haematologic toxicities was observed such as gastrointestinal, hair loss, infection, cutaneous hyperpigmentation, neurotoxicity, nephrotoxicity, and hepatotoxicity. The complete blood count was performed after treatment one week with repeated blood count and blood

chemistry before subsequent course. The toxicity was recorded according to WHO classification.

Response assessment and further treatment : Patients were evaluated by physical and gynaecological examination each month. Chest radiograph and pelvic ultrasound scan was used as necessary. Owing to the economic problem, the computer tomogram and tumour marker were not performed for the assessment. The clinical response was assessed using standard criteria of WHO.

The second look laparotomy was performed in the patient who achieved clinical complete response for evaluation of the pathological response. Partial response to chemotherapy in previous debulk surgery was treated by radical surgery and second course of chemotherapy. The pathological complete response patient was treated by oral cyclophosphamide 150 mg/day and 5-fluorouracil 300 mg/day for 5 days, every 4 weeks for 6 cycles as prophylaxis to prevent recurrence. The residual cancer post second look laparotomy was treated by second line chemotherapy or external radiation.

Statistical anaysis : The difference in toxicities, response rates between two arms was determined by chi-square test. Kaplan and Meier's method were used to estimate and compare survival.

Results

From October 1987 to October 1990, 48 patients entered this study, Patient characteristics and treatment were summarized in Table 1. There were no significant differences between the two arms of the study in age, performance status, stage of disease, surgical procedure, histological type, and grading. The optimal surgery could be performed only in few cases of both arms.

The response to treatment were summarized in Table 2. In arm A. 50% had complete response and 20% had partial response. In arm B, 37.5% had complete response and only 5% had partial response (P=0.1). Arm A achieved the negative second look laparatomy or pathological complete response in 77.7%, whereas in arm B the some was found in 76.9% (P=0.39). The recurrence after negative second look laparotomy were found in both arms (A=28.5% and B=20.0%) (P=0.39). The duration of recurrence occured after negative second look laparotomy 4.0 and 19.5 months of arm A, where as 8.0 and 9.0 months of arm B. In arm A one patient who had stage IV lesion was alive with out disease for 4-7 months Another patient expired with survival time 33.0 months one case in arm B expired in 12.0 months. All of the 12 cases in both arms who had pulmonary nodules or liver metastases expired with survival time of 5.0-60.0 months. The median survival time was

Table 1 *Patient characteristic and treatment*

Characteristics	CBP 300 mg/m² + CTX 500 mg/m² Arm A	CBP 400 mg/m² + CTX 500 mg/m² Arm B
Eligible for study	n = 20	n = 20
Age range (year)	28 - 75	37 - 63
mean	51.75 ± 13.73	49.1 ± 7.69
ECOG performance		
1	7 (35.0%)	6 (30.0%)
2	10 (50.0%)	12 (60.0%)
3	3 (15.0%)	2 (10.0%)
FIGO stage III A	2 (10.0%)	3 (15.0%)
B	1 (5.0%)	3 (15.0%)
C	8 (40.0%)	8 (40.0%)
IV	9 (45.0%)	6 (30.0%)
Surgical procedure		
Optimal surgery	3 (15.0%)	4 (20.0%)
Suboptimal surgery	7 (35.0%)	8 (40.0%)
Minimal surgery	10 (50.0%)	8 (40.0%)
Histological type		
Serous	11 (55.0%)	10 (50.0%)
Mucinous	6 (30.0%)	6 (30.0%)
Clear cell	3 (15.0%)	2 (10.0%)
Endometrioid	0	2 (10.0%)
Histological grade 1	5 (25.0%)	4 (20.0%)
2	8 (40.0%)	8 (40.0%)
3	7 (35.0%)	8 (40.0%)

Optimal surgery = hysterectomy, bilateral salpingo-oophorectomy, infra colic omentectomy (residual cancer ≤ cm.)
Suboptimal surgery = as optimal surgery but residual cancer > 1 cm.
Minimal surgery = partial resection of cancer or only biopsy (bulky residual cancer)

25.0 months (95% confidence interval : 10.2-48.2 months) for arm A, and 35.5 months (95% confidence interval : 17.6-52.4 months) for arm B. The median survival of the optimal surgery group was 78.0 months in arm A, and 53.0 months in arm B. The suboptimal surgery showed median survival 50.0 months in arm A, and 52.0 months in arm B. The minimal surgery group showed median survival 12.5 months in arm A, and 14.5 months in arm B. The Kaplan and Meier's survival curve were constructed in Figure 1, with 3 years survival 40.0% in arm A and 55.0% in arm B (P = 0.14).

Table 2 *Response of treatment*

	CBP 300 mg/m² + CTX 500 mg/m² Arm A	CBP 400 mg/m² + CTX 500 mg/m² Arm B	P-value
Clinical complete response	10 (50.0 %)	15 (75.0 %)	
Partial response	4 (20.0 %)	1 (5.0 %)	
Stable disease	2 (10.0 %)	1 (5.0 %)	0.1
Progressive disease	4 (20.0 %)	3 (15.0 %)	
Pathological complete response	7/9 (77.77%)	10/13 (76.92%)	0.39
Recurrence after negative second-look	2/7 (28.57%)	2/10 (20.0 %)	0.39
Median survival (months)			
Optimal surgery	78.0	53.5	0.29
Suboptimal surgery	50.0	52.0	0.91
Minimal surgery	12.5	14.5	0.79
Over all median survival	25.0	35.5	0.29
Three year survival	8/20 (40.0%)	11/20 (55.0%)	0.14

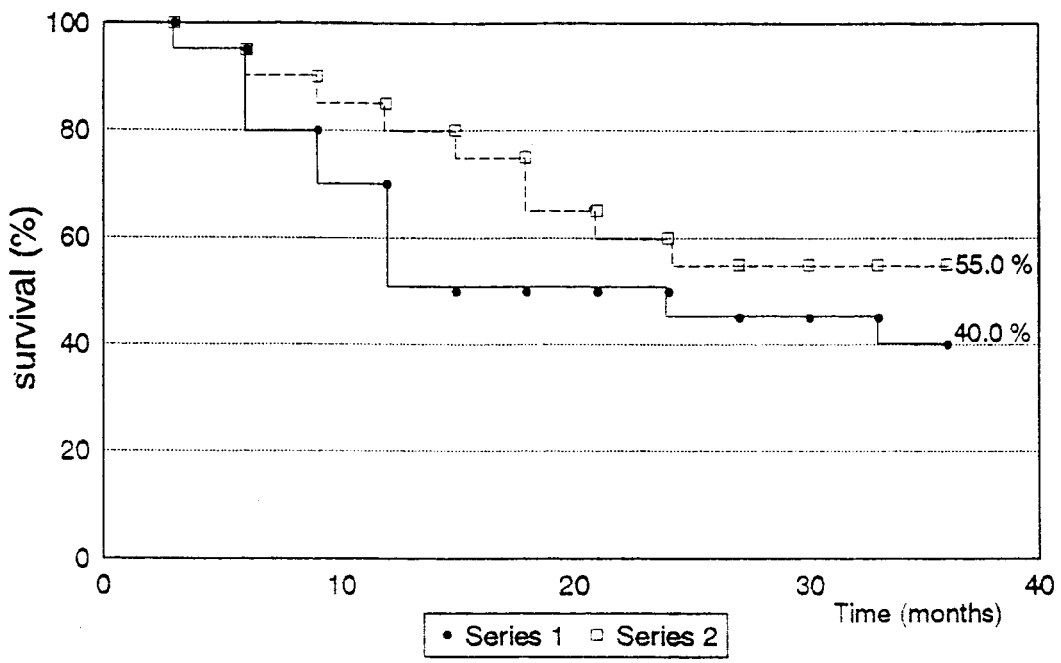


Fig. 1 Survival curve of the patients arm A (Series 1) and arm B (Series 2)

Table 3 *Toxic effects*

Toxic effects	Grade	Arm A (cases)	Arm B (cases)	P-value
1. Haematology				
Haemoglobin (gm/100 ml)				
8.0 - 9.4	2	7	8	
6.5 - 7.9	3	4	4	
< 6.5	4	2	4	
Transfusion required	-	6 (30.0%)	8 (40.0%)	
Leukocytes ($x10^3/mm^3$)				
3.0 - 3.9	1	6	8	all>0.05
2.0 - 2.9	2	4	6	
1.0 - 1.9	3	2	3	
< 1.0	4	2	2	
Platelets ($x10^3/mm^3$)				
75 - 99	1	2	3	
50 - 74	2	3	3	
25 - 49	3	3	3	
< 25	4	1	4	
Petechiae	-	1	1	
2. Gastrointestinal				
Nausea and vomiting				
Transient	2	7 (35.0%)	13 (65.0%)	-
Required therapy	2	1 (5.0%)	3 (15.0%)	
3. Nephrotoxic				
Creatinine >2 mg/100ml	-	0	0	-
4. Neurotoxic	-	0	0	-
5. Hair loss				
Minimal	1	14 (70.0%)	13 (65.0%)	
Moderate	2	5 (25.0%)	7 (35.0%)	-
Complete	3	1 (5.0%)	0	
6. Moderate infection	2	0	2	
7. Cutaneous hyperpigment	-	1	3	-

The toxicities of two arms were shown in Table 3. The haemoglobin levels grade 3-4, requiring blood transfusion was found in arm A 30.0% and in arm B 40.0%. The leukocytes count grade 2-4 caused delay of treatment, occurred in arm A 40.0%, and arm B 55.5%. These differences showed no statistical significance. The thrombocytopenia produced delay of treatment, was found in arm A 50.0% and arm B 65.0%, required the platelets transfusion in one case of each arm.

Nausea and vomiting grade 3 required therapy were found in arm A 5.0% and arm B 15.0%. There were no evidence of hepatotoxic, nephrotoxic and neurotoxicity. Both arms showed the similarity of mininal hair loss. The infection grade 2 occurred in only 2 cases of arm B. The cutaneous hyperpigmentation was found in arm A 1 case and 3 in arm B.

Discussion

The efficacy of combined carboplatin and cyclophosphamide in two different doses of carboplatin achieved the response rate of 70.0% and 80.0%, with clinical complete response of 50.0% and 75.0% respectively. The arm B showed slightly better in clinical complete response, median survival, and 3 years survival; but the pathological complete response and recurrent rate after negative second look laparotomy were similar. These outcome showed no significant differences, due to small sample sizes and minimal difference of carboplatin doses.

Few patients of both arms showed haematologic toxicity grade 4, which required the intensive treatment. Nausea and vomiting were easily managed by prophylactic antiemetic in both arms.

There were no evidence of hepatotoxic, nephrotoxic and neurotoxicity. The minimal to moderate hair loss showed spontaneous recovery after drugs discontinuation. This evidence showed the satisfactory results at a level of low toxicity. The carboplatin 300 mg/m^2 and cyclophosphamide 600 mg/m^2 for 6 cycles were previously reported in treatment for epithelial cancer stage II-IV diseases provided response rate of 66.7% and clinical complete response of 45.8% with median survival 24.0 months.[11] and other investigator used similar dose, reported a comparable median survival of 22.7 months.[12] Thus, the optimal dose for our patients should be carboplatin 400 mg/m^2 and cyclophosphamide may be increased to 600-700 mg/m^2, with good results, without hydration, low toxicity, It is suitable for out patient administration, with reasonable cost of therapy.

Acknowledgement

The authors wish to thank Ass. Prof. Ronachai Atisuk for statistical help, and Bristal Myers Squibb Thailand Ltd for partial support of paraplatin (carboplatin).

References

1. Ehrlich CE, Einhorn LH, Williams SD, and Morgan J. Chemotherapy for stage III,IV epithelial ovarian cancer with cis-dichlorodiammineplatinum, adriamycin and cyclophosphamide, a preliminary report. Cancer Treat Rep 1979; 63:281-288.
2. Ehrlich CE, Einhorn LH, Stehman FB, Roth LM, Blessing J. Response, "second look" status and survival in stage III-IV epithelial ovarian cancer treated by cis-dichlorodiammine platinum II (cis-platinum), adriamycin, and cytoxan. Gynaecol Oncol 1980;10:367-368.
3. Neijt JP, Ten Bokkel Huinink W, Van der Burg M, et al. Randomised trial comparing two combination chemotherapy regimens (CHAP-5 v CP) in advanced ovarian carcinoma. J Clin Oncol 1987;5:1157-1168.
4. Omura G, Ehrlich C, Blessing J. A randomised trial of cyclophosphamide, plus adriamycin with or without cis-platinum in ovarian carcinoma. Proc Am clin Oncol 1982;1:104.
5. Omura G, Blessing JA, Ehrlich CE, Miller A. Yordan E, Creasman WT. Homesley HD. A randomised trial of cyclophospha-mide and doxorubicin with of without cis-platin in advanced ovarian carcinoma. Cancer 1986;57:1725-1730.
6. Sessa C, Bolish G, Colombo N, Incalci MD, Mermillod B, Valente I, Mangioni C. Xexamethylmelamine, adriamycin and cyclophosphamide (HAC) versus dichlorodiammineplatinum, adriamycin, and cyclophosphamide (PAC) in ovarian cancer : a randomised clinical trial. Cancer Chemother Pharmacol 1985;14:222-228.
7. Senapad S, Neungton S, and Teerapagawong C. Cyclophosphamide, adriamycin, and cisplatinum in treatment of ovarian epithelial cancer stage III-IV. Siriraj Hosp Gaz 1987;39:61-65.
8. Calvert AH, Harland SJ. Early studies with cisdiammine-1, 1-cyclobutane dicarboxylate platinum II. Chemother Pharmacol 1982;9:140-147.
9. Curt GA, Grygiel JJ, Corden BJ, et al. A phase I and pharmacokinetic study of diammine cyclobutane dicarboxylate platinum NSC 241240. Cancer Res 1983; 43:4470-4473.
10. Albers D, Green S, Hannigan E, et al. Improved efficacy of carboplatin (Carbo+cyclop) vs cisplatin (CP), preliminary report of a phase III randomised trial in stage III-IV suboptimal ovarian cancer. Proc Am Soc Clin Oncol 1989;8:151.
11. Gurney H, Crowther D. Five year follow-up and dose delivery analysis of cisplatin, iproplatin or carboplatin in combination with cyclophosphamide in advanced ovarian carcinoma. Annals of Oncology 1990;1:427-433.

Survey of the Knowledge, Attitudes and Practice (KAP) Towards Infertility and Sexually Transmitted Diseases (STDs)

Verapol Chandeying,*
Roengsak Leetanaporn,*
Thitima Suntharasaj,
Patrick J. Rowe#

*Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkla, Thailand.
#Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, Geneva, Switzerland.

Abstract : *The Knowledge, Attitudes, and Practice (KAP) towards infertility and Sexually Transmitted Diseases (STDs) management among primary health care workers have been studied in 415 health workers of seven provinces in Southern Thailand. All participants completed a questionnaire on the level of health behavior change continuum : 1) awareness and concern 2) knowledge 3) false attitudes and 4) practice. Most of the health workers were already aware and concerned about infertility and STDs, including the general concept of treatment and prevention. The definition and what constitutes infertility require explanation and understanding as nearly one-third of the respondants had incorrect ideas. The knowledge and skills to cope with infertility and STDs, as well as individual education on safer sex and existing STDs associated or with increased risk of HIV infection, were not satisfactory. (Thai J Obstet Gynaecol 1995; 7:69-75.)*

Key words : KAP, infertility, STDs, primary health care,

In Thailand, the health delivery system consisted of provision of services which include mother and child health and family planning, communicable disease control, school health, health education, and distribution of government produced household remedies through the existing health infrastructure from the centre to province, district, and sub-districts. The primary health level consists of community hospitals and local health centres in the districts and sub-districts respectively.[1]

In developing countries, limited health resources have not allowed widespread Sexually Transmitted Diseases (STDs) clinical services to be

established.[2] Physicians mainly work in hospital settings, from the district level up to national reference of teaching hospitals, and these facilities cater to the health problems of only 10 to 30 per cent of the population.[3] Paramedics, with variable supervision by physicians, are dealing with STDs[4] and also providing infertility services.

Many countries have noted increased infertility as a consequence of the current STDs epidemic; and the number of countries with growing STDs problems are increasing.[5] Prevention of infertility caused by STDs depends on a multifaceted, community wide effort by clinicians and other health workers to control the spread of STDs and to prevent sequelae of existing diseases.[6]

The major concern is to assess the current status of STDs and infertility services, and to train non physicians to do much of the physicians' work with apparent safety and patient satisfaction.[7] The priorities are to develop a rational approach to the management of infertility at the primary and secondary health care levels, and developing and pilot testing a primary health care management scheme directed towards the identification and treatment of lower genital tract infection as a means of preventing subsequent female tubal occlusion.[8]

Data concerning the management of STDs at the primary health care level in Thailand are rare, and in order to plan and manage the problems of STDs and infertility in the community, the detailed information is essential. It is important to identify the relevant information about STDs and infertility which is used in the community (local health centre). Increasing national concern over the shortage of physicians in rural areas, the primary care is first contact, comprehensive in scope, continuous in duration, and offering the generalist rather than the specialist perspective.[9]

The objective of the study was to survey the knowledge, attitudes, and practice towards STDs and infertility and STD management amongst primary health care workers. Our belief is that this evaluation of infertility and STDs management will provide information for the health authorities, so that primary health services in this area can be improved.

Materials and Methods

The participants were the health officers of the local health centers and of the STDs units in seven provinces of Southern Thailand: Songkla, Pattani, Yala, Narathiwat, Stoon, Pattalung, and Trang province.

The investigators conducted an anonymous questionnaire which were self administered among 415 health officers. All participants completed the questionnaire about the level of health behavior change in their infertility and STDs management, including (1) awareness and concern (2) knowledge (3) false attitudes (4) practice. In addition, a number of demographic

questions were asked.

Results

The 415 individual contacted were distributed by provinces as follows: Songkla (47), Pattani (46), Yala (78), Narathiwat (87), Stoon (50), Pattalung (49), and Trang (58). The mean age of the health workers was 31.9 years (SD ± 6.6), and the mean duration of working was 10.9 years (SD ± 8.2). Most of them were female (85.5%), married (69.6 %), Thai (92.2%), and Buddhist (73.4)%. The distribution of the health workers' profession was midwife (33.9%), nurse (21.6%), public health (9.3%), and other (33.7%).

The health workers' awareness and concern about AIDS is given in Table 1. Most of the workers were already aware and concerned about infertility and STDs.

Most of the respondants (92.5%) had heard of the word "infertility", but only 10.6% were capable to give a precise definition of "infertility". More than one fourth of them (28.4%) understood that the meaning of "infertility" was the same as "sterility". They understood the following conditions could result in infertility: congenital defect(s) of sex organs (76.1%), vaginal atresia or imperforate hymen (62.4%), cervical obstruction (86.5%), tubal occlusion (93.0%), endocrinopathy (90.6%), chronic illness (76.3%), faulty spermatogenesis (83.3%), faulty sperm transmission (83.6%), and faulty semen (67.4%). In addition, they were knowledgeable that various microorganisms can cause sexually transmitted diseases: bacteria (86.7%), viruses (84.3%), fungi (85.5%), and protozoa (72.2%).

Table 1 *Awareness and concern responses about infertility and STDs among the health workers (N=415)*

	Infertility No. (%)	STD No. (%)	P value *
Awareness response :			
- it is a health problem	337 (81.2)	388 (93.4)	S
- it is preventable	219 (52.7)	393 (94.6)	S
- it is communicable	14 (3.3)	365 (87.9)	S
Concern response :			
- feel your work is responsible towards, infertility and STDs	315 (75.9)	388 (93.4)	S
- ever sought more information about it	337 (81.2)	395 (95.1)	S
- ever discussed about it with your co-workers	294 (70.8)	360 (86.7)	S
- had in mind that history taking about this condition is useful	376 (90.6)	394 (94.9)	NS

* S = significant P < 0.05

Table 2 *Understanding the concept of STD treatment and prevention (N=415)*

Items	No. (%)
Treatment of STD	
- appropriate antibiotic to each disease is the best choice of therapy	358 (86.2)
- some diseases should include treatment of his/her sexual partner	401 (96.6)
- the treatment of the sexual partner can prevent "ping-pong" phenomenon	341 (82.1)
Prevention of STD	
- the condom is the best way to prevent STDs	363 (87.4)
- they should advise the patients to use the condom	366 (88.1)
- they should advise the patients to be examined when STDs symptoms occur	409 (98.5)
- they should have a blood test for some STDs in the high risk behavior populations	408 (98.3)
- they should advise the patients to avoid promiscuity	406 (97.8)
- they should advise the patients to use "safe sex"	406 (97.8)

Nearly two third (64.0%) of the health workers realized that all of the possible symptoms could present as STDs in females (increased vaginal secretion, offensive discharge, yellowish discharge, itching of the vulva, vulva ulcer or lesion, and lower abdominal pain), whereas 17.8% identified some of these items. In comparison, only 50.3% realized the symptoms associated to the STDs in males (dysuria, urethral discharge, and ulcer or lesion of the penis), and nearly one third of them (30.8%) identified some of these items. However, most of them were aware of STDs treatment and prevention as shown in Table 2.

As it can be seen in Table 3, the false perceptions increased to nearly one third of replies; infertility is caused by hormonal contraception (31.0%), infertility is caused by promiscuity (34.4%), and STDs is caused by sexually exposure only (38.0%).

Regarding to the general management of the infertile couple, most of primary health care workers (91.3 %) advised the couple to have an opinion from a doctor, and nearly half of them (54.9%) referred the patients to a provincial hospital. As regards to the STDs patients, 74.9% adviced the patients to have an opinion from a doctor, and 61.5% of

Table 3 *False perceptions about infertility and STD (N=415)*

Items	No. (%)
Infertility	
- infertility is caused by promiscuity	143 (34.4)
- infertility is caused by hormonal contraception	129 (31.0)
- infertility is caused by husband or wife alone	44 (10.6)
- infertility is caused by marital infidelity	27 (6.5)
- infertile couples should consult their priest or imam	15 (3.6)
- infertility is caused by supernatural being	10 (2.4)
- infertile couples should take native treatment	8 (1.9)
- infertility is caused by witchcraft	3 (0.7)
STD	
- STDs are caused by sexually exposure only	158 (38.0)
- STDs do not occur in children	61 (14.1)
- STDs can be prevented by antibiotic prophylaxis	61 (14.1)
- STDs can be prevented by immediately cleansing with antiseptic solution after sexual intercourse	44 (10.6)
- STDs can be aggravated by some kind of food or fruits	38 (9.1)
- STDs can be prevented by taken urinary antiseptics	18 (4.3)
- STDs can be cured by intercourse with virgin partner	10 (2.4)

Table 4 *Actual practice of infertility and STD among health workers*

Items	Infertility No. (%)	STD No. (%)	P value *
Usually take a history from the patients	294/415 (70.8)	320/415 (77.1)	NS
Number of patients < 5 per month who they usually consulted for the problem case	327/415 (78.8)	350/415 (84.3)	NS
- doctors	91/148 (61.9)	103/203 (50.7)	NS
- colleagues	9/148 (6.0)	20/203 (9.8)	NS
- other	15/148 (10.2)	32/203 (15.7)	NS
- not specify	33/148 (22.3)	48/203 (23.6)	NS
Results of their treatment			
- most of the patients had a good outcome	37/260 (14.2)	93/337 (27.5)	S
- some of the patients had a good outcome	93/260 (35.7)	202/337 (59.9)	S
- most of the patients had a poor outcome	130/260 (50.0)	42/337 (12.4)	S

* S = significant $P < 0.05$

them referred the patients to the community hospital.

Individual patient health education compatible with the health workers' concept of STDs prevention was carried out with the exception of "safer sex". They gave the details and recommended that the patient should have an HIV test in only 64.3%.

Discussion

This study describes the existing STDs and infertility management in Southern Thailand among primary health care delivery. The awareness and concern, and the understanding the concept of treatment and prevention among STDs and infertility are satisfactory (Table 1). However, the actual knowledge and professional skills to manage the problem in STDs and infertility efficiently are still inadequate (Table 2-4). Coordination and cooperation between concerned organizations needed in development of the curriculum and setting the training components. The aim is to strengthen the existing health workers in the cognitive, skill, and attitude developments. Thus, they would provide welcome hale for overworked physicians and improved access of health care services. There was concern, however, that health workers with relatively brief clinical training would not be able to provide good quality patient care, even when closely supervised by physicians.[10,11]

In Thailand, even if the introduction of the physician's assistant results in easier access to medical services at decreased cost, the assessment of the effect of the physician's assistants has been relatively little effort. The mechanism for assuring an acceptable standard of performance or to measure the quality of medical care shoul be developed and delivered. Clinical algorithms for STDs and infertility would be considered to developed to screen those patients who need to be referred to specialists in provincial hospitals or centres for sophisticated investigations. The algorithms system provides instructions for gathering and interpreting data. The ability to follow these instructions correctly may reflect throughness and reliability. It is not only the measurement of the accuracy of the observations made by the physician's assistant but also is the responsibility of the physician's assistant who examines the patients.[12]

Although many important questions about clinical algorithms can not be answered at present, it seem to be one possible use of intervention to provide many patients with access to a defined standard of medical care.[13] In general concept, the quality assurance of the medical services comprised 1) standard in personnel 2) standard in facilities and support 3) standard in services (STDs and infertility) and 4) standard in service cost. To acheive the optimal goal, closed observation and monitoring are required.

The peripheral laboratory so need to provide a simplified and

rational approaches to STDs and infertility, and strongly encourage to be used in the primary health care system where the patients come into contact with a health worker for the first time. It is expected that the health worker will have access to a simple microscope which would permit STDs and infertility screening or even diagnosis such as stained smear for identification of STDs pathogens, azoospermia, and post coital test etc.

Conclusion

This study has shown that the quality of primary health care service in STDs and infertility given by health worker was inadequate to cope the prevention and management of STDs and infertility. Selected intervention in STDs and infertility implementations would be considered. The recognition and strengthening the health workers may be possible answer, so need in quality improvement in STDs and infertility practice.

Acknowledgements

We would like to express our gratitude to the Task Force on the Prevention and Management of Infertility, Special Programme of Research, Development and Research Training in Human Reproduction, the World Health Organization for the financial support of the research.

References

1. Deesawadi P. Thailand. In: Azurin JC, ed. Proceeding of the 10th South East Asian Medical Information Centre Seminar Diseases Surveillance in Primary Health Care. SEAMIC, Tokyo, 1983:76-92.
2. Cates W Jr, Meheus AZ. Strategies for development of sexually transmitted diseases control programs. In: Holmes KK, Mardh PA, Sparling PF, Weisner PJ, eds. Sexually transmitted diseases. New York: McGraw-Hill, 1990:1023-1030.
3. Meheus AZ, Piot P. Provision of services for sexually transmitted in developing countries. In: Oriel JD, Harris JRW, eds. Recent advances in STD. Edinburgh: Churchill Livingston, 1986:261-272.
4. Arya OP, Bennett FJ. Role of the medical auxiliary in the control of sexually transmitted diseases in a developing countries. Br J Vener Dis 1976;52:116-121.
5. Sherris J, Fox G. Infertility as a public health problem. Population Reports, Series L No.4, Population Information Program, Baltimore, 1983.
6. Kramer DG, Brown ST. Sexually transmitted diseases and infertility. Int J Gynaecol Obstet 1984;22:19-27.
7. Sox HC. Quality of patient care by nurse practitioners and physician's assistants: A ten-year perspective. Annals Int Med 1979; 91:459-468.
8. Rowe PJ, Farley TMM. Prevention and management of infertility. Research in human reproduction. World Health Organization, 265-286.
9. Berg AO. The primary care physician and sexually transmitted diseases. In: Holmes KK, Mardh PA, Sparling PF, Weisner PJ, eds. Sexually transmitted diseases. New York: McGraw-Hill, 1990: 1095-1098.
10. Miles DL. Physician's assistants: the evidence is not in. N Engl J Med 1975; 293:555-556.
11. Baker AS. Primary health care by nurse. N Engl J Med 1974;290:282-283.
12. Sox HC, Sox CH, Tompkins PK. The training of the physicians's assistants. N Engl J Med 1973;288:818-824.
13. White KL, Murnagham JH, Gaus CR. Technology and health care. N Engl J Med 1972;287:1223-1227.

Pelvic Tuberculosis with Cervical Involvement: A Case Report

Kovit Pimolpan MD,
Suwanit Therasakvichya MD,
Pichai Charoenpanich MD,

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

Abstract : *Pelvic tuberculosis is seldomly found in female. The involvement of uterine cervix is rare with no particular specific feature. The macroscopic appearance gives a first impression of cancer. A cervical biopsy with histological study leads to diagnosis and then anti - tuberculous treatment may be administered There is no need for surgery only in drug resistant case. The authors reported a case of pelvic mass and hypertrophic growth of the cervix with histology Compatable with tuberculosis. The outlook was good after anti-tuberculous drugs were carried out. (Thai J Obstet Gynaecol 1995;7:77-80.)*

Key words : *tuberculosis, pelvic , cervix*

Genital tuberculosis is caused by Mycobacterium tuberculosis that produces a granulomatous lesion involving upper and occasionally lower genital tract structure. Pelvic infection is usually a consequence of dissemination of primary disease or spreading from visceral organs. However cervical involvement is rare[1]. The following case illustrated pelvic tuberculosis with cervical involvement,without evidence of pulmonary infection.

Case report

A 22-year-old primipara woman, was admitted to Siriraj Hospital with the chief complaint of having pelvic pain and vaginal spotting. She experienced spontaneous complete abortion at 16 week gestation two years ago. After that she used no contraception and has been in good health. Five months prior to admission she developed amenorrhea and increased vaginal discharge with no other symptom. She continued working and stopped two days before admission due to pelvic pain and bleeding per vaginam.

On physical examination, she was fair, hyposthenic built, weighed 38 kilograms, and had low grade

fever. Palpable irregular rubbery mass about 20 week gestation was detected in lower abdomen (Fig.1). Pelvic examination showed hypertrophic growth of the uterine cervix 4 cm in diameter with contact bleeding (Fig 2). The uterus could not be separately palpated from that mass. Ultrasonography revealed echogenic mass packing in pelvic cavity at left side of normal sized uterus and at the right side showed hypoechogenic area which was consistent with bowel loops as shown in plain film abdomen. No abnormality was detected on rectal examination. Complete blood counts , blood urea nitrogen, fasting blood glucose, electrolytes, VDRL, anti HIV, urine analysis and chest x-ray were all within normal limits.

Carcinoma of the uterine cervix with pelvic metastasis was suspected and cervical biopsy was performed. Histological study revealed necrotic tissue and area of tubercle formation containing large histiocytes with multinucleated giant cells, that was consistent with tuberculosis (Fig. 3). Acid fast stain for Mycobacterium tuberculosis was positive. The patient had positive tuberculin skin test but negative sputum examination for acid fast stained bacilli. The patient was treated with anti - tuberculous drugs which included isoniazid 300 mg daily,ethambutol 800 mg daily, rifampicin 450 mg daily and pyrazinamide 1000 mg daily.

One month later,she gained weight 2.5 kg and still had amenorrhea but no pelvic pain. Pelvic mass was decreased in size (about 6 cm in diameter). The cervix had nearly normal appearance (Fig.4). She was

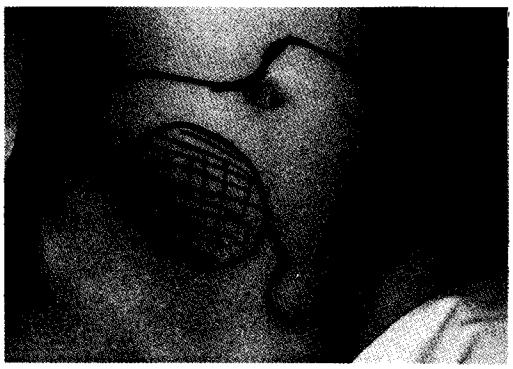


Fig. 1 Irregular pelvic mass (before treatment)

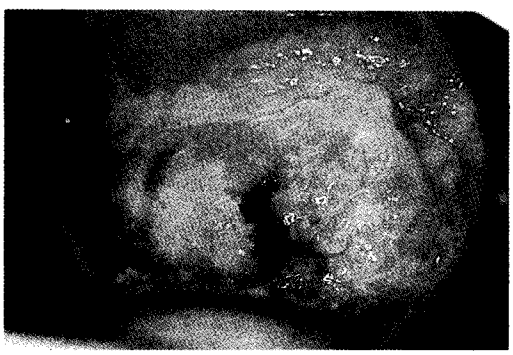


Fig. 2 Hypertrophic growth of cervix (before treatment)

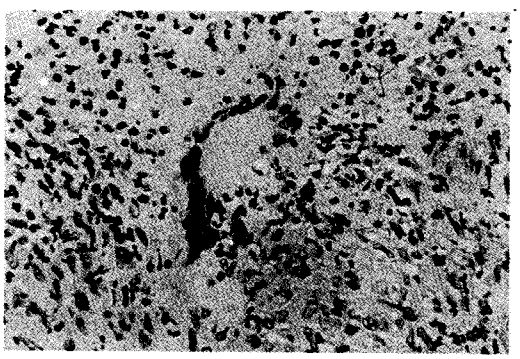


Fig. 3 Necrotic tissue and area of tubercle formation containing large histiocytes with multinucleated giant cells (X 200)

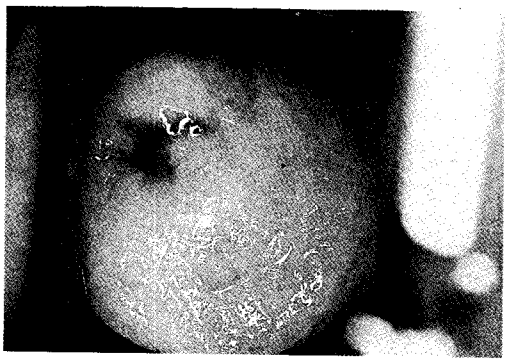


Fig. 4 Nearly normal cervix (after treatment)

given 6-month course of combined anti-tuberculous drugs without surgical intervention and no adverse effect.

Discussion

Pelvic tuberculosis is usually a consequence of hematogenous dissemination of primary pulmonary disease. It may produce salpingitis, dysmenorrhea, amenorrhea, infertility or otherwise asymptomatic[2]. An exceedingly rare lesion that generally secondary follows widespread pelvic involvement is tuberculois of cervix. Cervical tuberculosis as a primary lesion can be transferred during sexual activity by a male with genital tuberculosis[3]. The diagnosis is rarely made by gross inspection only, the confirmation by biopsy is mandatory[4]. Our case illustrates malignant like appearance of uterine cervix associated with irregular pelvic mass in amenorrheic woman. The cervical histology characterized by multiple tubercles with multinucleated giant cells has to be differentiated from fungal infection,foreign body giant cell granuloma reaction suture, crystals, and sarcoidosis[5,6]. The unequivocal diagnosis requires the demonstration of acid fast Mycobacterium tuberculosis by Ziehl-Neelsen stained sections, as in this case, otherwise by cervical tissue culture[7].

Current therapy should include the use of combined agents such as isoniazid,ethambutol,rifampicin and pyrazinamide. Surgical removal of pelvic structure may be indicated only in nonresponsed case[8] Tuberculosis causes extreme tissue damage, and the prognosis of fertility is poor so that follow up evaluation and counselling of the patient is essential.

References

1. Grossman JH III , Larsen JW Pelvic infections. In: Kase NG, Weingold AB, Gershenson DM. Principles and Practice of Clinical Gynecology 2nd. ed New York:Churchill Livingstone Inc., 1990:592-593.
2. Good JT, Iseman MD, Davidson PT, et al: Tuberculosis in association with pregnancy. Am J Obstet Gynecol 1981;140:492.
3. Chatterjee PK,Sundar-Rao CH:Non-pulmonary tuberculosis with special reference to pathological aspects. J Indian Med Assoc. 1979;72:245.
4. Schaefer C:Tuberculosis of female genital tract. Clin Obstet Gynecol 1970;13:965.
5. Evans CS,Goldman RL, Klein HZ, Kohout ND:Necrobiotic granulomas of the uterine cervix. A probable postoperative reaction. Am J Surg Pathol 1984; 8:841.

6. Nogales-Ortiz F, Tarancon I, Nogales FF:The pathology of female genital tuberculosis. Obstet Gynecol 1979;53: 422
7. Ferenczy A,Winkler B Benign disease of the cervix. In:Kurman RJ Blaustein's Pathology of the Female Genital Tract 3rd ed. New York: Springer-Verlag Inc, 1987:163.
8. Chatane A, Rhrab B, Jirari A, Ferhati D,Kharbach A, Chaoui A Hypertrophic tuberculosis of the cervix:three cases. J Gynecol Obstet Biol Reprod 1992;21: 424-427.

Surgical Treatment of an Extensive Lesion of Lymphangioma Circumscriptum of the Vulva

Berrin Acar MD,
Yakup Erkan Erata MD,
Atakan Topuz MD,
Turhan Uslu MD,
Cemal Posaci MD,
Tülay Canda MD.[1]

Department of Obstetrics and Gynecology Dokuz Eylul University, Medicine faculty Izmir- Turkey.
Department of Pathology[1]
Dokuz Eylul University, Medicine faculty Izmir - Turkey.
Correspondence to: Yakup Erkan ERATA 110. Sok. Simsaroglu sitesi Banu apt. No: 2/18 Goztepe-Izmir Turkey.

Abstract : *A 52 years old postmenopausal female patient was admitted because of inability to have coitus due to a lesion in her vulva. It was found that despite various modes of therapy given to the patient who had had the same complaint for ten years, the lesion had enlarged. The couple also stressed that they had problems in their normal social life because of their impaired sexual life. The clinical appearance suggested the presence of a case of lymphangioma circumscriptum. This diagnosis was confirmed by biopsy studies. Total vulvectomy was performed with the removal of the deep subcutaneous fat tissue. No reccurence was observed over a follow up period of two years. The present study aims to demonstrate that extensive surgical treatment might be successful in cases in terms of preventing reccurence, when this lesion infrequently occurs in the vulva in an extensive fashion; and the patient gains the ability of having normal sexual life. (Thai J Obstet Gynaecol 1995;7:81-84.)*

Key words : Lymphangioma circumscriptum, vulva, surgical treatment.

Lymphangiomas are cases of malformation rather than true neoplasms. They are quite rare and are mostly of congenital nature occuring immediately after birth or in the first year of life[1,2]. These are three types, lymphangioma circumscriptum being the most infrequent one[1,2]. The term lymphangioma circumscriptum was first used by Morris in 1889[3]. The lesion manifests itself in the subepidermal vesicles. There are lymphatic cisterns deep set in the subcutaneous tissue. These cisterns are develop-

mental anomalies and are not connected to the deep lymphatic system. However, they are connected to the dermal lymphatics along dilated lymphatic vessels in cases of lymphangioma circumscriptum. The vesicles forming under the skin are saccula dilatation of superficial lymphatics[1]. They are translucent, pale and 3-4 mm in diameter. When they burst open a mucoid fluid comes out. This fluid contains proteinaceous material and lymphatic fluid. Infection and increment in the size of the lesion are frequently seen. Lymphangioma circumscriptum has been reported to occur most frequently in the upper trunk, the neck, the axillae and the tongue[1,2]. Various modes of therapy have been performed in cases of lymphangioma. In local cases, cryotherapy and cauterization have proved effective[2]. In local and diffuse cases, radiotherapy has not only proven ineffective, but irradiation has given rise to some side effects. Surgical intervention has been attempted, yet recurrences are frequent[1,2]. The process has been found to recur at sites of primary suture and in the graft, in cases where the defect was grafted[3]. It was Whimster who first suggested that recurrence would be the rule, unless the deep cisterns were totally removed[4].

In 1977, Jordan et al observed a case of lymphangioma circumscriptum of the heep, that the lesion did not recur after total removal of the skin and the subadjacent subcutaneous fat tissue[3].

A case of vulvar lymphangioma circumscriptum, with such extensive spread as to prevent coitus, is unique in the literature. Thus, at the outset, the authors were unable to predict the extent to which they might be successul. Furthermore, occurence of the lesion at such advanced age was an additional feature of the case.

Case Report

A 52 years old postmenopausal female patient was admitted to our clinic complaining of a lesion in the vulva. She stated that due to this lesion, she was so uncomfortable, unable to wear underclothes and unable to have coitus. It was found that this complaint had been present for ten years. Previously when the lesion was small in a local state, it was cauterized and various therapies were given in an effort to prevent infection. Yet, it was found that the lesion had progressed and spreaded to the entire vulva. On clinical examination, (Fig.1) large groups of flat papillomas and infected pseudovesicles (3-5 mm in diameter) were observed in the vulvar skin. The skin was thickened and deformed. Biopsy was obtained following antibiotic therapy for infection. Pathological examination revealed that the epidermis had become thinner at some places with the papillae having become elongated and acanthosis in some areas. There were sections of enlarged lymphatic vessels immediately underneath

the epithelium. These were surrounded by the endothelium and contained proteinaceous fluid and lymphocytes in their lumina. A diagnosis of lymphangioma circumscriptum was established. Total vulvectomy was carried out consisting of the removal of deep subadjacent subcutaneous tissue. No postoperative complications occured. Pathological examination confirmed a clinical diagnosis of lymphangioma circumscriptum elephantiasis. The microscopic appearance of the lesion is shown in (Fig. 2).

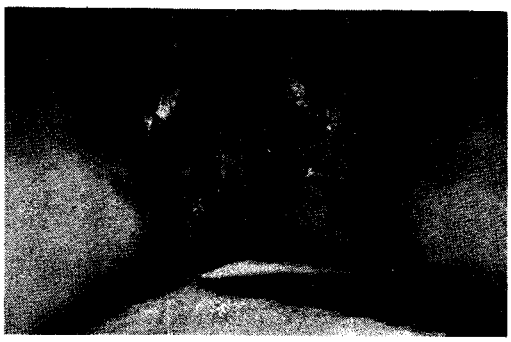


Fig. 1 Macroscopic appearence of the lesion.

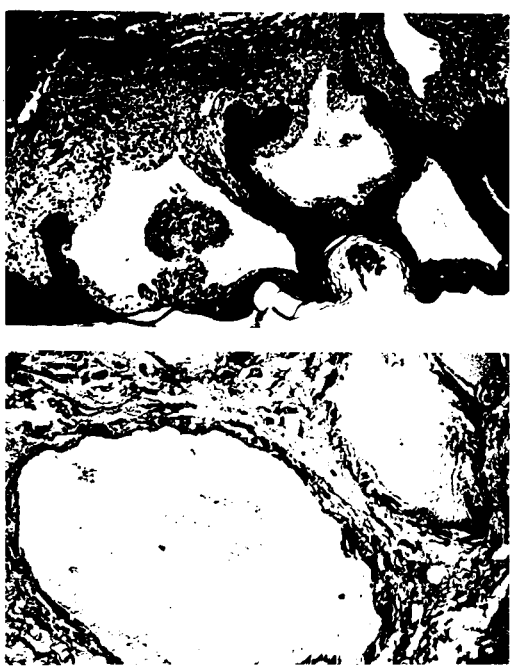


Fig. 2 Microscopic appearence of the lesion.

The patient was followed up for more than two years. No recurrence was seen. The patient felt extremely comfortable upon the elimination of the lesions. She and her husband stated that they had regained the ability to have coitus.

Discussion

Lymphangioma circumscriptum is a rarely encountered disease and is usually considered to be of congenital origin. It is seen at birth and within the first postnatal year. A very small number of cases have been reported in the adult age group[1,2]. In the present case, the quite advanced age of the patient and hindrance of coitus due to pain are striking features. Inability to have coitus constituted a great problem for the family.

Cases of lymphangioma circumscriptum of the vulva in the literature in which various therapy models were applied, resulting in the different sequences. In 1989, Abu Hamad et. al.[5] reported a case which partial vulvectomy was performed with no recurrence in the short follow-up period of six weeks. In 1991, Johnston et. al.[6] reported two cases of lymphangioma circumscriptum of the vulva in which extensive vulvar surgery were per-

formed and recurrences were observed in the follow-up period in both of them. Later in 1992, Mu-rugan et. al.[7] reported a case in which simple vulvectomy, was performed and recurrence was observed after nine months and the lesion was excised again. Our treatment was based on the previous studies byJordan[3] and Whimster[4] who described cases of lymphangioma circumscriptum occuring in other parts of the body. We performed total vulvectomy with the removal of deep subadjacent subcutaneous fat tissue containing the cisterns. Recurrence was not observed over a follow-up period of 2 years. There was no keloid formation. Restoration of the patients comfort and regaining of the possibility of comfortable coitus seem to be favorable results of the present study.

Among the cases of vulvar lymphangioma circumscriptum reported, two cases were described following radiotherapy for squamous cell carcinoma of the cervix[8,9], and another case reported by Sood et al.[10] had a history of surgery and treatment of pulmonary tuberculosis and scars near the left cervical and vaginal region. In the present study, there was no history of surgery or severe extragenital disease.

In conclusion, when simple vulvectomy is indicated in cases of vulvar lymphangioma circumscriptum, total removal of the subadjacent subcutaneous fat tissue, as far as the fascia, is recommended in order to prevent recurrence. We suggest that this therapy model should be the first choice in the treatment of vulvar lymphangioma circumscriptum.

References

1. Fitzpatricle TD, Eisen AZ, Wollf K, Freedberg IM, Austen KF. Diseases of lymphatics. Dermatology General Medicine. 1979;83:735-736.
2. William V. Lymphangioma. Clinical dermatology. 1973;2:1-4.
3. Jordan PR, sanderson KV, Wilson JSP. Surgical treatment of lymphangioma circumscriptum: A case report. Brit J Plast Surg. 1977;30:306-307.
4. Whimster IW. The pathology of lymphangioma circumscriptum. Brit J dermatol. 1976;94:473.
5. Abu Hamad A, Provencher D, Ganjei P, Penalver M. Lymphangioma circumscriptum of the vulva: case report and review of the literature. Obstet Gynecol. 1989 Mar; 73(3 Pt 2):496-499.
6. Johnston TL, Kennedy AW, Segal GH. Lymphangioma circumscriptum of the vulva. A report of two cases. J reprod Med. 1991 Nov.;36(11):808-812.
7. Murugan S, Srinivasan G, Kaleelullah MC, Rajkumar L. A case report of lymphangioma circumscriptum of the vulva. Genitourin. Med. 1992 Oct; 68 (5):331.
8. Fisher l, and Orkin M. Acquired Lymphangioma (Lymphangiectasis) Arch Dermatol. 1970;101:230-234.
9. La Palla J, Foucar E, Leshin B, Whitaher D and Anderson B. Vulvar Lymphangioma Circumscriptum: A rare complication of therapy for squamous cell carcinoma of the cervix. Gynecol Oncol. 1985;22:363-366.
10. Sood M, Mandal AK, Ganesh K. Lymphangioma circumscriptum of the vulva. J Indian Med Assoc. 1991 Sep;89(9): 262-263.